Behavioral and Physiological Effects of Hydrocarbon Exposure on Selected Arctic Invertebrates

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Executive Summary

A series of experiments were carried out in 1982 and 1983 with the purpose of defining the behavioral responses and the dynamics of hydrocarbon uptake and clearance in three species of benthic invertebrates, Strongylocentrotus droebachiensis, Serripes groenlandicus and Mya truncata, following exposure to Lago Medio crude oil. The dosing protocols were designed to a) correlate with the 1981 BIOS experimental spill program and b) to reproduce low level, long term hydrocarbon exposure which may be anticipated from point source offshore oil spills such as a leaking tanker or ruptured pipeline or from a blowout.

The experimental procedure involved exposure to dispersed crude oil in a flow-through seawater system on site at Cape Hatt, N.W.T. Oil was added to test systems as an oil oil/Corexit 10/1 mix at concentrations of 50 ppb to 500 ppm. A series of three sequential 6 hour exposure regimes were also tested to simulate Bay 9 spill conditions at 10, 100 and 5 ppm and Bay 10 spill conditions at 0.5, 5.0 and 0.2 ppm. In addition in 1983, a low level, long-term exposure experiment was carried out using a regulated oil seawater flow-through system. In all instances, samples were taken for hydrocarbon analysis of water and tissue during the dosing period and at set intervals during clearance.

Behavioral responses of Mya truncata to dispersed oil as determined during the 1982 field experiments were confirmed during the 1983 tests. Among the behavioral effects noted in the 1983 repetition of the Bay 9 and Bay 10 spill simulations were ostial closure and siphon retraction and at higher exposures a loss of responsiveness to mechanical stimulus. Prolonged exposure to low level hydrocarbon concentrations of 50 ppb and 500 ppb did not elicit any behavioral stress response.

In both 1982 and 1983 tests, S. groenlandicus exhibited sensitivity to oil by ostial closure and siphon retraction. In addition, general emergence out of the sediment occured when exposed to single dose concentrations greater than 100 ppm. Higher doses resulted in shell gaping and foot extrusion following an initial response of extreme valve closure which resulted in shell margin damage. Exposure to 500 ppm resulted in a loss of stimulus response and latent lethality.

S. groenlandicus exposed to the simulation of field spill conditions (the Bay 9 and Bay 10 simulations) showed pronounced behavioural responses. Exposure to low initial doses enhanced the response to the middle dose and resulted in a delayed recovery. Ostial closure and emergence during the 50 ppb chronic exposure test were biphasic and latent. Within 11 days of the chronic exposure S. groenlandicus was strongly narcotized and non-responsivie. Exposure to 500 ppb also caused delayed ostial closure and a delayed but almost uniform emergence from the sediment. In this case the bivalves were less narcotized.

S. droebachiensis proved to be sensitive to almost all exposure levels in 1983; this sensitivity was manifested by the early loss of adherance to the substrate and loss of the ability to retain debris cover. Spine rigidity was characteristic of urchins exposed to low concentrations while spines tended to droop at higher exposure levels, at some instances during the clearance period. Retraction of tube feet was common. Narcosis was manifested by a loss of tube foot and spine responsiveness. The dose response relationship demonstrated in 1982 was further documented by stat stical analysis in 1983. Exposure to the Bay 9 spill simulat ons in 1982 resulted in a 1 of the behavioral responses tested and this within the first 6 hours of exposure. The Bay 10 spil simulations resulted in the most pronounced response during the middle exposure sequence; i.e. during the 6-12 hour higher level exposure.

Differences in behavioral responses and hydrocarbon uptake were noted for S. droebachiensis exposed to Bay 9 and Bay 10 simulations. The total absence of spine rigidity, the delayed onset of most of the stress related behavioral indices and the lower hydrocarbon body load of 1983 test organisms exposed to the Bay 9 spill simulation indicate a more oil resistant population in 1983 than in 1982.

Only the 500 ppb chronic exposure resulted in the modification of normal behaviour patterns. The reaction was mild and delayed.

Separate tests were carried out to assess the effects of Corexit alone; to differentiate sensitivities of small and large individuals and to test for the behavioral responses of Serripes groenlandicus to oiled sediment. Exposure of the three test organisms to Corexit resulted in a mild and short lived stress response, while no differences were noted in the behavioral responses of large and small animals exposed to a single static exposure.

S. groenlandicus exposed to oil sediment did not behave differently from the control populations. A limited hydrocarbon load was accumulated by the oiled sediment population.

All three species took up petroleum hydrocarbons at a level which was dose related S. droebachiensis continued to load soft tissues with hydrocarbon transferred from the external body surface even during the clearing periods.

The behavioral data are interpreted in the context of individual survival, of potential for hydrocarbon loading, and generally in relation to the BIOS experimental spill results for the biota in Bays 9, 10 and 11.

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Sample collection from the field was carried out by divers from LGL Ltd.

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Introduction

In the course of the four years of research undertaken as part of the Baffin Island oil spill project (BIOS) a considerable body of knowledge has been acquired not only on the ecology and distribution of arctic benthic populations, but also on their sensitivity and ability to recover from exposure to petroleum.

Observations made during the 1981 field spills demonstrated that bivalves and urchins were affected by the dispersed oil released into the test bays. This was characterized by the emergence of bivalves from the sediment, the unusual attitudes of echinoderms, and narcosis of bivalves. Confirmation of these visual observations was obtained during the first phase of a two year study which was designed to document the behavioral effect of petroleum exposure and to define the dynamics of hydrocarbon uptake, storage and clearance characteristics under controlled laboratory experiments. The behavioral component of the study conducted in 1982 provided information on the relative sensitivity of the three test species to dispersed oil, and on their potential for survival. During the 1983 phase, spill relevant dosing sequences were repeated to further document the characteristics of hydrocarbon uptake and deputation in the test species, and to correlate these with the behavioral effects described in 1981 and 1982.

A study on differential hydrocarbon loading of critical body tissues of animals exposed to chronic doses of dispersed oil was carried out concurrently with the study of behavioral effects of the low level exposure. The gut and visceral mass of bivalves were isolated, their total hydrocarbon burden evaluated and characterized.

A sub-sample of *S. groenlandicus* and *M. truncata* exposed to spill relevant Bay 9 and Bay 10 sequential exposures, and to chronic exposures, yielded information of filtration, respiration, assimilation rates, scope for growth ratios as well as levels of enzymatic activity. Results of this study are described elsewhere (Gilfillan and Vallas 1984).

Methods and Materials

Location

The field portion of this study was carried out at Cape Hatt in Eclipse Sound, N.W.T. (72° 28'N, 79° 50'W), at the location of the 1980 - 1983 Baffin Island Oil Spill study. On-site laboratory experiments were designed to investigate the behavioral responses of three selected species of benthic invertebrates to chemically dispersed crude oil, and to detail the dynamics of hydrocarbon uptake, storage and clearance. These tank studies were conducted on the shores of Cape Hatt during the period of August through early September of 1982 and 1983.

Experimental Design

Structural Design

A flow-through system was constructed on-site to provide a number of 50 L aquaria with a continuous flow of seawater at a rate of approximately 100 ml/ min per tank. Seawater was drawn from an offshore intake at a water depth of 8 m (high tide), 1 metre above the bottom.

During the short term exposure periods, the flow-through system was temporarily interrupted in experimental and control tanks and individual circulators were used to recirculate the total water volume at a rate of 8 l/rein. In 1983 all tanks were covered with sheet glass during the dosing period.

To achieve chronic exposure conditions, the flow-through system was modified. After an initial static dose, a continuous supply of the 50 and 500 ppb solution was ensured through the automatic injection of a stock solution of the dispersed oil into the main seawater flow to each of the exposure tanks. Temperature and salinity were verified regularly throughout the experimental period and found to be 4° ± 1°C and 24 °/oo ± 3 °/oo and 6° ± 1°C and 26 °/oo ± 3 'loo in 1983. These conditions were in the range of temperatures and salinities recorded in the offshore water where the test species were collected. The holding facility was maintained at natural light conditions.

Of the three test species, two were the filter-feeding bivalves, Mya truncata and Serripes groenlandicus, and another surface deposit-feeder, the green sea urchin Strongylocentrotus droebachiensis. All three species were collected by divers in

the waters of Milne Inlet at 7 m depth off the western shore of Ragged Island, a site near Cape Hatt but removed from potential oil contamination during the 1981 experimental spill. The test animals were transferred to the field laboratory, where they were acclimated in the flow-through seawater system for at least 7 days prior to the exposure studies. Mortalities during this acclimation period were less than 3% in either year. Fresh seaweed (Laminaria sp.) and broken shell material was supplied regularly to the urchins during the pre-exposure period. S. groenlandicus was kept in a 10 cm layer of washed and sifted coarse beach sand, while both M. truncata and S. droebachiensis were held without sediment in the test tanks.

1982 test species were of a medium size range Mya truncata were 4.6 ± 0.4 cm, S. groenlandicus 4.3 ± 0.6 cm and urchins 4.6 ± 0.5 cm. One test series also used small and large individuals (M. truncata 2.7-4.4 ± 0.3 cm, S. groenlandicus 2.7 -5,1 ± 0.4 cm and S. droebachiensis 3.0-5.4 ± 0.3 cm). Holding densities in 1982 were calculated not to exceed field densities as identified in Cross and Thompson 1981 and were limited to 20 individuals of medium size for each species per 50 L aquaria.

1983 test species were also of a medium size range. Mya truncata were 4.0 ± 0.3 cm, S. groenlandicus, 4.2 ± 0.5 cm and S. droebachiensis were 4.5 ± 0.6 cm. Although 1983 holding densities were greater than 1982 levels they still fell within the range of densities observed in the field (Cross and Thompson 1981). Requirements for additional tissue hydrocarbon and enzyme analysis required the addition of 18 additional individuals per tank for the 7 day acclimation period and for the first 18 hours of all dosing sequences.

Exposure protocol

Dispersed oil concentrations were prepared by dilution of a stock oil/dispersant mixture. This was prepared by vortex stirring of the BIOS-stock aged Lago Medio crude oil with Corexit-9527, (1:10), in seawater, as a 25,000 ppm solution. All concentrations are expressed on a basis of weight of crude oil.

In 1982 eight experimental conditions were tested, as outlined in Table 1. In all instances, the dosing procedure was similar, with concentrations, exposure times and number or size of animals being varied in accordance with the intent of the test. Single six hour exposures were carried out to assess dose-responses or experimental variables, sequential exposures (three at 6 hours each) were designed to correlate with conditions recorded in Bays 9 and 10 after the field spill

TABLE 1 DOSING PROTOCOL 1982, 1983

Experimental Condition	Concentrations**	Exposure Time (h)	Clearance Time (wks)	Test Organisms*
Single exposure	0,0.5,5,50,100,500	6	1	MYA SER STR
Sequential exposure Bay 10 simulation	0.5/5.0/0.2	6 each	1	MYA SER STR
Sequential exposure Bay 9 simulation	10/100/5	6 each	1	MYA SER STR
Single exposure (covered and uncovered comparisons)	250	6 6	1 1	STR STR
Sequential exposure (covered and uncovered comparisons)	10./100./5	6 each	1	MYA SER STR
Single exposure (size comparisons)	250	6	1	MYA SER STR
Single exposure (predation test)	250	6	1	SER
Corexit exposure	50	6	1	MYA SER STR
Sequential exposure Bay 10 simulation	0.5/5.0/0.2	6 each	3	MYA SER STR
Sequential exposure Bay 9 simulation	10/100/5	6 each	3	MYA SER STR
Chronic exposure	50ppb	2 wk	0	MYA SER STR
Chronic exposure	500 ppb	2 wk	0	MYA SER STR
Oiled sediment	30	1 wk	0	SER

* SER - Serripes groenlandicus
MYA - Mya truncata
STR - Strongylocentrotus droebachiensis

** Concentrations in ppm except as noted

experiment. Corexit was tested alone at one concentration. Other experiments were carried out to differentiate the effect of a probable partial loss of volatiles from the dispersed oil during the exposure period.

A clearance period of seven days was used to assess recovery. Controls for each species were monitored concurrently with the exposure experiments.

During the 1983 field program, the sequential dosing regimes were repeated. Although the exposure period remained the same, i.e. 18 hours; the clearance period was extended to three weeks to assess the rate of clearance and to verify the tissue hydrocarbon concentrations recorded in the 1981 oil spill field samples. The tests species were also exposed to chronic doses of dispersed crude oil concentrations of 50 ppb and 500 ppb for a period of 14 days.

This chronic exposure was achieved by a flow-through exposure system which relied on the automatic and metered injection of a stock solution of dispersed oil into the main water flow of each of the exposure tanks.

The stock solution of 50 ppm for the 50 ppb delivery and 600 ppm for the 500 ppb was kept sealed, agitated and renewed daily. A flow meter regulated the oil/Corexit stock solution into the main seawater stream which itself was regulated to deliver 100 ml of test solution per minute per tank. This was dispersed into a starting concentration of 50 ppb or 500 ppb and was maintained for 14 days as a flow-through system. Fluorometric analysis of water samples from the experimental tanks compared nominal to actual concentration .Daily fluctuations were less than 20%.

Controls for each species were monitored concurrently with the dosing experiments.

All experimental tanks in 1983 were covered by sheet glass to minimize possible loss of some of the volatile components of the dispersed oil.

A third dosing protocol was established in 1983 to investigate the behaviour of S. groenlandicus exposed to oiled and unoiled sediment. Beach sediment was dried and its water retention potential determined. A seawater oil and Corexit mix was prepared to a 30 ppm concentration and added to the dried sediment. The system was flushed for 24 hours and the acclimated S. groenlandicus introduced to control aquaria and oily sediment aquaria. Behavioral observations of time to burial, degree of locomotion, ostial attitude were recorded.

Sampling scheme: During both field seasons, water samples were taken at regular intervals for each exposure system and analysed by real time fluorometry.

In 1982, 50 ml water samples were taken from each exposure system at the beginning, at 2 hour intervals and at the end of each exposure sequence. Additional samples taken during the clearance period when the tanks were being flushed with clean seawater were analysed by real time fluorometry. Selected water samples taken during the 1982 exposure tests were analysed by gas chromatography.

Water samples from the Bay 9 sequential dosing experiment were collected for 1 species in 1983 at the beginning and end of each of the exposure sequences for headspace analysis and total gas chromatographic analysis.

Samples were taken for tissue hydrocarbons at the end of the exposure periods (6 or 18 hrs) and at 7 days. During the second phase of the study a large number of additional samples were collected at intervals duplicating the 1981 field sampling scheme both during dosing and clearance periods. The samples were analysed by uv fluorescence or by gas chromatography.

Soft tissues of five individuals of each species were removed and pooled as a single sample for each analysed time period. The tissues were wrapped in aluminum foil, sealed in whirl-paks and frozen.

Tissue hydrocarbon were sampled for all three species at fixed interval during the chronic exposures for uv fluorescence or GC analysis. At t=18hrs, 7 and 14 days the gut and visceral mass of 10 individuals of both M. truncata and S. groenlandicus were isolated for GC analysis. The pooled gut-tissue consisted of the oesophagus, stomach and intestine which was isolated from the gill, and mantle tissue. The test and body mass of five urchins were also separately analysed.

Water sediment and tissue samples were collected at t=7 days from both control and experimental S. groenlandicus and analysed by GC.

Controls were assessed concurrently for each of the test species and exposure tests and selected samples were analysed by GC. All glassware and instruments used for hydrocarbon residue samples were pre-rinsed and dried with high purity hexane-methylene chloride solvent.

Analysis

Seawater collected from the exposure tanks in 1982 and 1983 were analyzed by real time fluorometry. The fluorometric method used a Turner model 240 flow-through fluorometer, calibrated and blank adjusted before and during use. Dispersed Lago Medio crude was used for calibration. All readings were carried out in the linear range of the fluorescence calibration, at times necessitating dilution of test samples with sea water. Reproducibility of duplicate samples was found to be 5% or better.

1982 and 1983 tissue hydrocarbon residues were analyzed by Gas Chromatography as follows. Extraction of the samples was carried out according to protocols previously used in the BIOS core program. These included aqueous caustic digestion followed by solvent (hexane) extraction (Boehm 1982). Fused silica capillary GC (GC^2) of an aromatic hydrocarbon fraction (f_2) obtained through alumina/silicic acid fractionation of the total extract was used (Boehm et al 1982). Naphthalene (N), methyl (C_1) naphthalenes, ethyl/dimethyl (C_2) naphthalenes and trimethyl (C_2) naphthalenes (C_3) were quantified. The ratio of C_3N to total aromatics and in turn the ratio of total aromatics to total oil as determined by a combination of electrobalance gravimetric measurement on total oil and aromatic (f_2) fraction and total C_3N peak area: total f_2 peak area, was used to convert C_3N to "total oil" values for neat oil. As each sample was analyzed by GC^2, the total amounts of N, C_1N, C_2N, C_3N, and alkylated benzenes were obtained directly from GC^2 trace. The C_3N: total oil conversion was used to arrive at the "total oil" values reported for the animals.

Tissue hydrocarbon residues analysed by uv fluorescence were all subjected to an alumina column chromatographic cleanup procedure to remove a large fraction of interfering lipid material prior to UV/F analyses. Three extract dilutions were quantified relative to an "aged" Lagomedio oil standard analyzed at the same range and attenuation as the actual samples. The spectral response (intensity) of the samples at 350 nm, corresponding roughly to the alkyl naphthalene band, was measured. The response for the three dilutions were converted to ug oil using a Lago Medio calibration curve, taking into account dilution volume. "Total petroleum concentrations" were obtained by dividing by the wet weight of sample extracted. The sediment samples were solvent extracted using the methanol, dichloro-methane-methanol analyses for "total petroleum" or for high resolution capinary gas chromatographic analyses to determine compositional information.

A number of behavioral responses were recorded and quantified. The choice of particular behavioral indices of petroleum effect was based mainly on extensive observations of the three species during the acclimation periods. Only those indices judged to be both quantifiable and significant to the survival of the individual were selected. Characteristics of the index responses are detailed in Table 2.

Quantification of these responses was based on the proportion of the test population showing a change as related to exposure concentration and elapsed time. A standard point of comparison was the time at which 50% of the starting population showed a response (ET50). Recovery was assessed as that point in elapsed time at which the proportion of animals showing the response had returned to pre-exposure levels. The values for ET50 and recovery time were obtained from data plots made for each species and exposure test, comparing percentage of test population showing the response to elapsed time.

The data from 1982 tests here combined by statistical analysis and graphed 1983 data was also analysed and plotted. Best fit curves were drawn for both sets of plots and revised ET50 and recovery times were identified. Tables 5, 6, 7, 8, 9, 10 summarize 1983 and combined 1982 values.

Behavioral changes in addition to quantified responses were also described. Such changes tended to be less consistent, or could not be observed to involve at least 50% of the test population at any concentration.

TABLE 2 BEHAVIOURAL RESPONSES OF MYA TRUNCATA, SERRIPES GROENLANDICUS AND STRONGYLOCENTROTUS DROEBACHIENSIS EXPOSED TO CRUDE OIL DISPERSIONS

Target Species	Behavioral Responses	Description
Strongylocentrotus droebachiensis	direct observations	
	cover	shell fragments and pieces of algae cover test on aboral surface
	attachment	attachment to glass of aquarium floor and sides by tube feet
	spine droop	depression of spine downward along test surface
	spine rigidity	'pincushion" orientation and loss of flexibility
	curling of tube feet	aboral tube feet showing curline of distal ends
	retraction of tube feet	aboral tube feet not existent
	stimulus response	
	tube feet	tube foot retraction following mechanical stimulus laterally on test
	spine	re-orientation towards source of mechanical stimulus laterally on test
Mya truncata	direct observations	
	ostial closure	closure of both inhalent and exhalent siphons
	siphon retraction	retraction of siphon to 1/2 or less of normal extended length
	stimulus response	
	siphon	siphon retraction following mechanical stimulus to siphon
	mantle	retraction of mantle and closure of shell following mechanical stimulus of mantle edge
Serripes groenlandicus	direct observation	
	buried	burial to depth where ostia are exposed
	ostial closure	closure of one or both ostia
	mantle gape	mantle edges not closed and shell gaping
	stimulus response	
	siphon	siphon retraction following mechanical stimulus to siphon

Statistical Analysis

Initial exploratory data analysis showed the data could not be handled by parametric statistical tests. Problems arose with the distribution and a lack of replicated experimental data. After pooling of the 1982/1983 control data it became apparent that there were insufficient data to construct a 95% confidence interval to compare experimental exposures and get meaningful results.

In all exposure protocols the organisms showed a binomial distribution in their responses. The binomial distribution is applicable to samples of any size from populations in which objects occur in two classes only. Therefore, the data were analysed using Fisher's Exact Test, a nonparametric technique, to analyse categorical (discrete) data. When samples come from discrete data, small sample sizes, and the two independent random samples fall into one or the other of two mutually exclusive classes then one can use the Fisher Exact Probability Test.

2 X 2 Contingency Table

		+	total
group 1	A	B	A+B
group 2	c	D	C+D
total	A+C	B+D	N

(from Siegel, 1956)

In this case group 1 and 2 would be control and experiment and (-) and (+) would be the behavioral response eg. ostia open, ostia closed. The probability of observing this distribution is given by;

$$p = \frac{(AB!(C+D)!(A+C)!(B+D)!}{N!A!B!C!D!}$$

(from Siegel, 1956)

The null hypothesis is that the two groups respond in the same way. The alternate hypothesis is that they differ; group 2 responds more or less than group 1 (ie. a two tailed test).

For all exposure protocols the Two Tailed Fisher Exact Test was performed to test the difference between the control and experimental groups for each observed behaviour. The significance level was chosen at alpha = 0.05. For the 250 ppm covered/uncovered experiment covered was tested against uncovered. In an attempt to see if there was any difference in the response of all single doses at the same time period (all or none effect) the Chi Squared Test for independence was used. This test was not valid in most cases due to the cell sized (A,B,C, or D from figure above) having expected counts of less than 5 in 20% of the cells. Therefore Fisher Exact's Test was performed on all possible pairs of concentrations to see if the responses where concentration dependent. The same was done with both the 1982 and 1983 sequential exposure protocols where Bay 9 was compared against Bay 10, covered vs. uncovered and small vs. large in the size experiment for S. droebachiensis.

The data from the initial dosing period of the experiment comparing size differences at 250 ppm did not allow statistical comparison. When the data were available (hr 24) there was no difference in the responses. Therefore these data were not analysed for S. groenlandicus and M. truncata. The control data for the initial twenty hour sampling periods of the 1982 sequential dosing experiments for S groenlandicus and M. truncata did not allow statistical-comparison. Therefore, the control data from the 1983 sequential dose experiment were substituted for the first twenty hours of 1982.

All data were entered in the computer as number responding, number not responding by the concentration (Bay for sequential) and the time. The Statistical Analysis System (SAS) frequency procedure was used to calculate the percentages responding, the significance values from the Fisher Exact Test and the Chi Squared Test. All statistical manipulations were analysed by time. The plots were generated by the SAS plot procedure.

The morphometric data were analysed using the general linear model procedure, a SAS procedure for an analysis of variance on unbalanced data sets. The mean values for size were analysed using the Student-Newman-Keuls multiple range test with a significance value of p 0.5 to test for a difference in size with respect to time and to concentration or bay.

RESULTS

Exposure Concentrations

In 1982, a comparison of nominal and fluorometry-measured concentrations of crude oil hydrocarbons in various test systems showed deviations of as much as 50% from the expected starting concentrations. The direction of the deviation was usually upward. This was shown for both the single exposure test (Table 3) and the sequential exposure tests (Table 4). In all instances, the measured concentrations declined over the 6h periods of static recirculated exposure to an average of two thirds of starting concentrations. Subsequent calculations of concentrations expressed as ppm/h averaged over the 6h exposures indicated that the exposure concentrations were very similar to the nominal concentrations.

Problems in supply logistics and mechanical performance of instrumentation precluded measurement of hydrocarbons in water during the sequential exposure systems in 1983. The methodology was identical to that used in the 1982 tests and therefore concentrations are expected to be within the range reported for 1982.

In the case of the chronic exposures, nominal concentrations were verified in the field for the 500 ppb exposure while water samples from the 50 ppb test were stored and later extracted for fluorometric analysis. A daily volumetric account of the stock solution yielded a performance control for the flow meters.

Behavioral Responses

Mya truncata

Behavioral responses of *M. truncata* to the presence of oil were inconsistent, particularly at lower concentrations. Ostial closure appeared to be the most sensitive response but a threshold concentration for both ostial closure and siphon retraction was not apparent (Table 5, Figure 1). Loss of response capability to mechanical stimulus manifested by a retraction of the mantle was recorded for concentrations of 50 ppm and more. However, even this response was inconsistent and often latent. Overall recovery of the animals was rapid at low level exposure even at times during the dosing period.

TABLE 3 PETROLEUM HYDROCARBON CONCENTRATIONS IN WATER OF SINGLE TEST SYSTEMS ' COMPARING NOMINAL CONCENTRATIONS TO VALUES DETERMI NED BY REAL-T I ME FLUOROMETRY AND BY GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED SAMPLES (1982)

Nominal Concentration[a] (ppm)	Time[b] (hours)	Test (ppm) Species[c]	Measured Concentration (ppm) Fluorometry	Gas Chromatography
0	0.1	MYA	nd	
		SER	nd	
		STR	nd	
	12	MYA	nd	
		SER	nd	
		STR	nd	
0.5	0.1	MYA	0.70	
		SER	0.55	
		STR	0.00	
	6	MYA	nd	
		SER	0.40	
		STR	0.40	
	12	MYA	nd	
		SER	nd	
		STR	nd	
5.0	0.1	MYA	8.0	
		SER	8,7	
		STR	7.8	
	6	MYA	6.0	
		SER	6.4	
		STR	6.8	
	12	MYA	nd	
		SER	nd	
		STR	nd	
50	0.1	MYA	60	
		SER	60	
		STR	64	
	6	MYA	26	26
		SER	22	
		STR	23	
	12	MYA	nd	
		SER	nd	
		STR	nd	
100	0.1	MYA	140	
		SER	128	
		STR	136	
	6	MYA	86	75
		SER	80	86
		STR	78	44
	12	MYA	nd	
		SER	nd	
		STR	nd	
250	0.1	MYA	380	(295)[d]
		SER	350	(133)
		STR	310 (350)	320 (183)
250	6	MYA	225	(96)
		SER	225	(86)
		STR	200 (225)	269 (1531
500	6	MYA	310	307
		SER	290	
		STR	290	
	12	MYA	nd	
		SER	nd	
		STR	nd	

a Calculated as three dilutions of Lago medio crude-Corexit stock dispersions, presented sequentially.

b Elapsed time from start of three Sequential exposure periods, each of 6-hours duration.

c MYA - Mya truncata; SER - Serripes groenlandicus; STR - Strongylocentrotus droebachiensis

d ncv - tanks not covered during exposure period; cv - tanks covered during exposure period.

nd= not detectable, - no data collected

TABLE 4 PETROLEUM HYDROCARBON CONCENTRATIONS IN WATER OF SEQUENTIAL TEST SYSTEMS' COMPARING NOMINAL CONCENTRATIONS TO VALUES DETERMINED BY REAL-TIME FLUOROMETRY AND BY GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED SAMPLES (1982)

Nominal Concentration[a] (ppm)	Time[b] (hours)	Test(ppm) Species[c]	Measured Concentration (ppm) Fluorometry ncv[d]	Fluorometry cv	Gas Chromatography ncv	Gas Chromatography cv
0.5/5/0.2	0.1	MYA	0.40		nd	
		SER	0.35			
		STR	0.40			
	6	MYA	0.20		nd	
		SER	nd			
		STR	nd			
	6.2	MYA	10		nd	
		SER	5.0			
		STR	6.5			
	12	MYA			nd	
		SER	5.0			
		STR	4.0			
	12.2	MYA	1.2		nd	
		SER	1.1			
		STR	0.65			
	18	MYA	1.0		nd	
		SER	1.0			
		STR	0.80			
10/100/5	0.1	MYA	12, 6.5	7.5	nd	nd
		SER	10, 8.0	8.0		
		STR	10, 7.5	8.0		
	6	MYA	8.5, 6.5	6.5	nd	nd
		SER	10, 5.0	6.2		
		STR	6.5, 5.0	5.0		
	6.2	MYA	150, 80	65	73,159	130
		SER	150, 80	75		
		STR	100, 90	65		
	12	MYA	95, 65	50	110,171	105
		SER	70, 50	50		
		STR	65, 50	50		
	12.2	MYA	10, 4.0	6.5	nd	nd
		SER	8.5, 5.0	6.5		
		STR	8.5, 5.0	6.5		
	18	MYA	7.0, 4.0	4.0	nd	nd
		SER	7.0, 3.7	4.0		
		STR	7.5, 3.7	4.0		

a Calculated as three dilutions of Lago *medio crude-Corexit* stock dispersions, presented sequentially.

b Elapsed time from start of three sequential exposure periods, each of 6-hours duration.

c MYA - *Mya truncata*; SER - *Serripes groenlandicus*; STR - *Strongylocentrotus droebachiensis*

d ncv - tanks not covered during exposure period; cv - tanks covered during exposure period.

nd= not detectable, - no data collected

Exposure to high concentrations required longer recovery times. During 1983, ET 50 and recovery times were re-evaluated from the statistically derived graphs of 1982 data (Figures 2, 3, tables 5, 6). The data derived from these graphs are in agreement with ET 50 values drawn from the 1982 raw data.)

Although Corexit at 50 ppm elicited all four of the behavioral changes, in some cases after exposure, the time for recovery of normal behaviour pattern was much shorter. Within 1 day of clearance, M. truncata exhibited a normal behaviour.

Exposure to chronic levels of 50 ppb and 500 ppb oil/Corexit mix did not alter the behaviour of M. truncata from that observed in control animals.

Results from the sequential exposure experiment indicated that responses were delayed relative to single exposures. These responses often did not occur during the period of highest concentration as is exemplified in the 100 ppm phase of the Bay 9 sequence (Table 6). Recovery times were also increased with respect to the equivalent single exposures (eg. 100 ppm single dose) . Bay 10 exposure did not affect the normal behaviour pattern of this species.

Neither size differences nor the effect of a sealed exposure tank could be differentiated in the M. truncata tests. This species was the most stable of all test organisms with respect to size.

Other behavioral changes were observed to occur incidentally to oil exposure. At low concentrations, or early in the exposure periods, the diameters of siphon ostia tended to be increased. Occasional extensions of the foot were more common. Elongation of the siphon was observed at high concentrations, especially late in the post-exposure period. No lethality was recorded.

Serripes groenlandicus

Of all behavioral indices monitored for this species, ostial closure appeared to be the most responsive. Closure occurred at all concentrations and was the only response observed in S. groenlandicus exposed to 50, 10, 5, 0.5 ppm oil exposures. (Table 7, figures 4, 5, 6, 7, 8). Ostial closure showed a concentration response from O to 100 ppm in that the time required to affect closure decreased with increasing concentration. This dose response was discontinued for concentrations greater than 100 ppm where ostia were closed for 50% of the population only after several hours. Recovery time was correspondingly delayed with increased concentration.

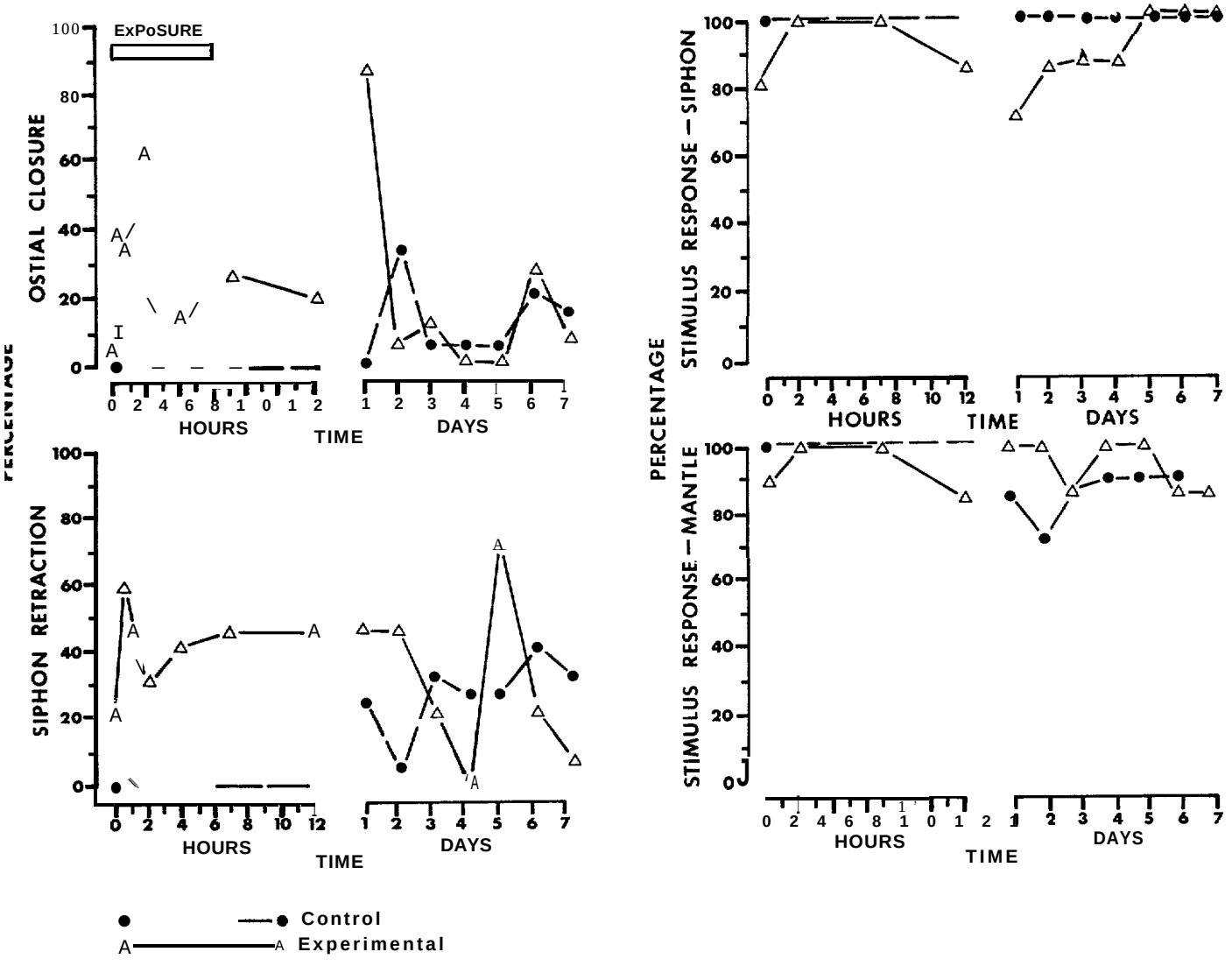
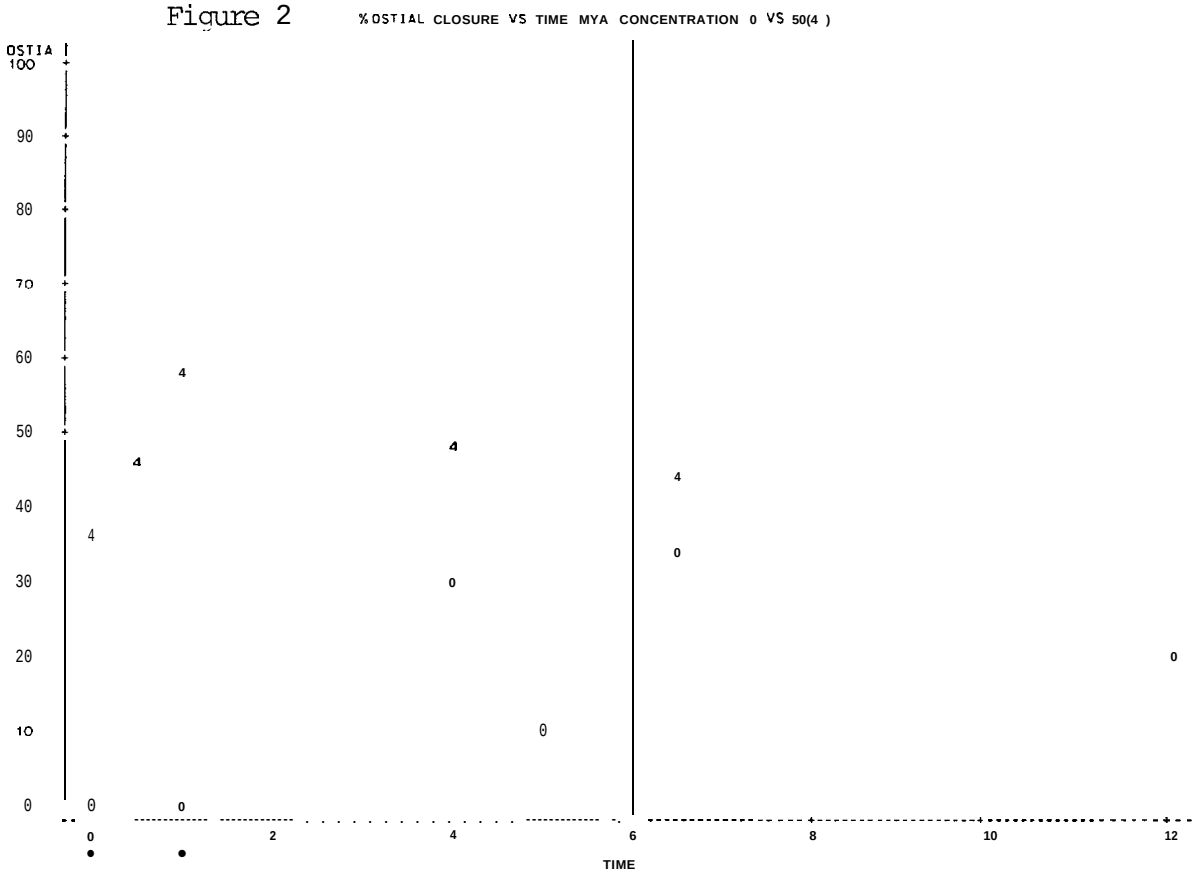
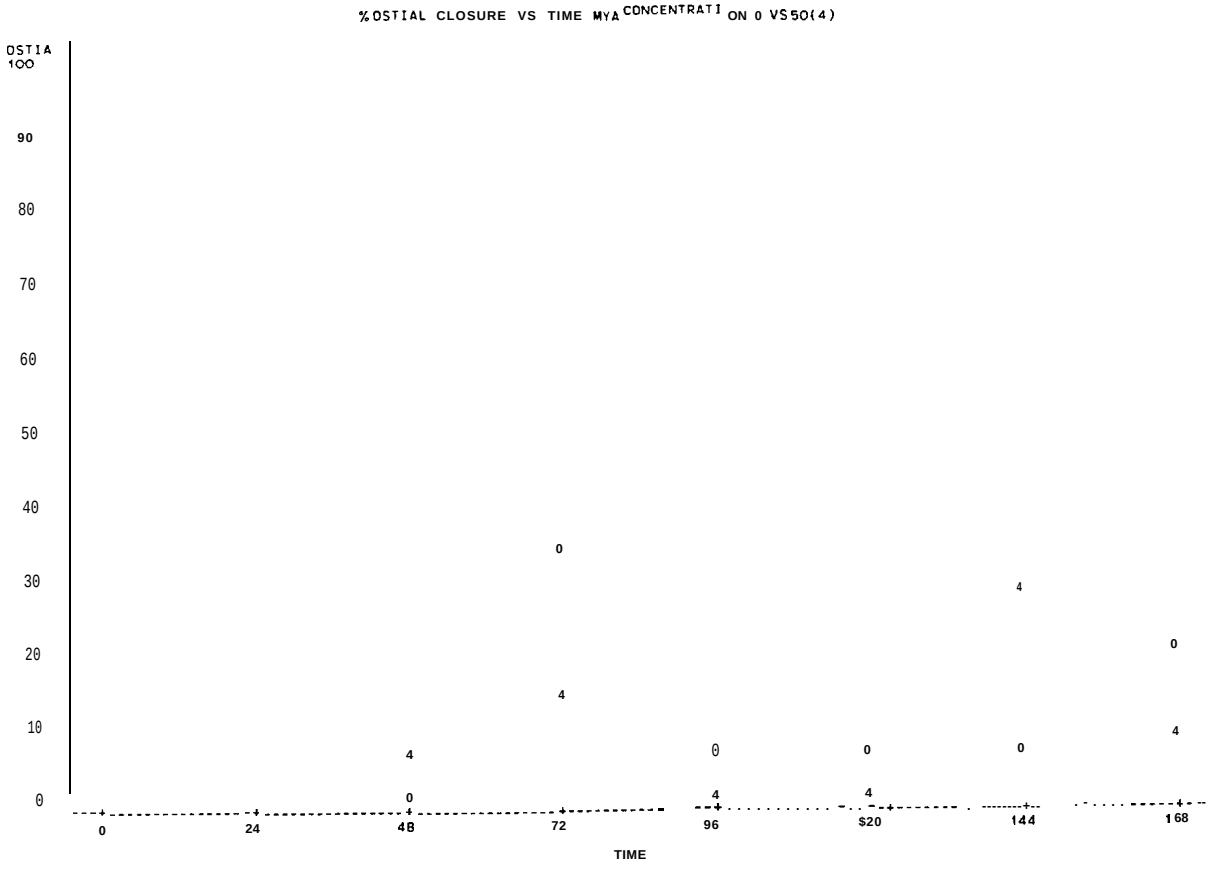


Figure 1

Selected behavioral responses of **Mya truncata** to **50** ppm dispersed oil

Figure 2

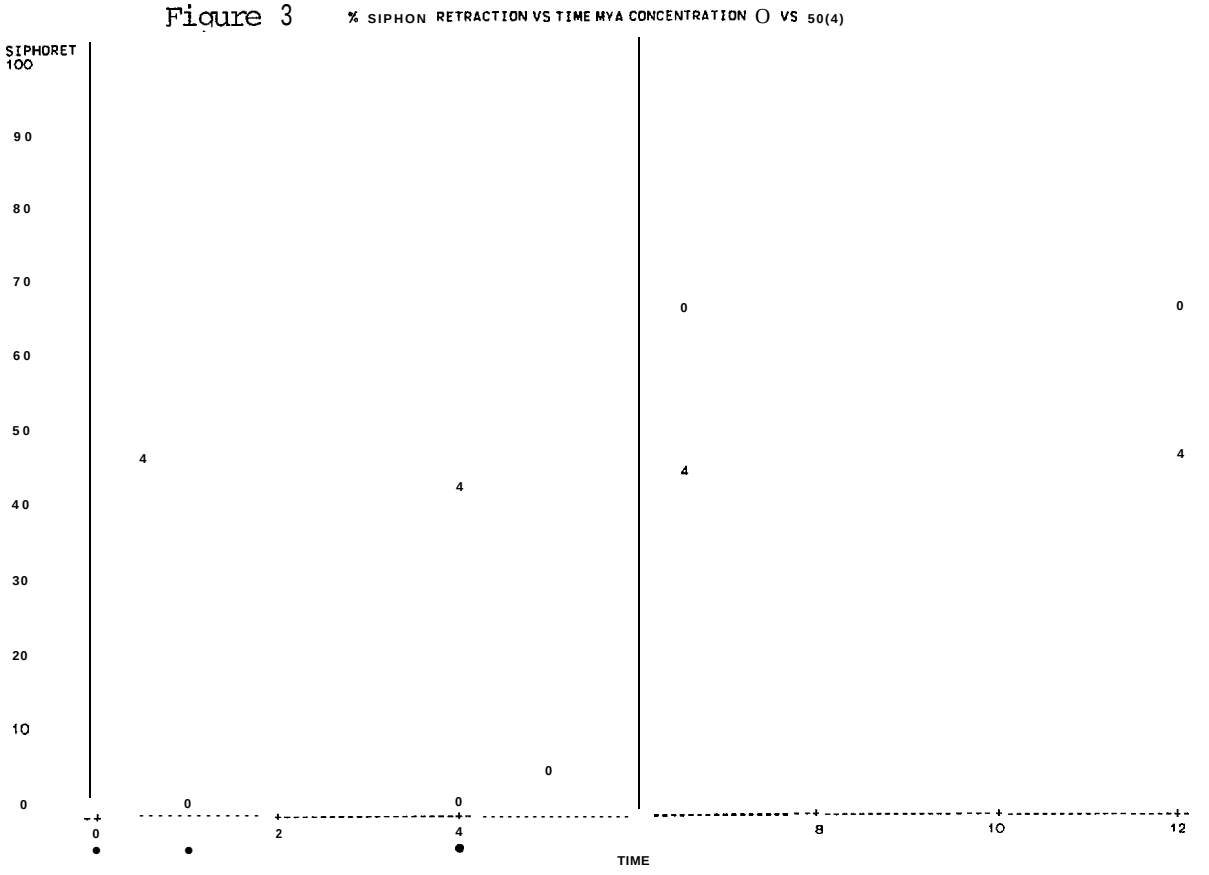




NOTE 1 (JBS HAD MissInG VALUES OR wERE OUT OF RANGE

"Significantly different values ($p \leq 0.05$)
Missing obs. or obs. out of range = missing value in one of the comparisons at that time

Figure 3



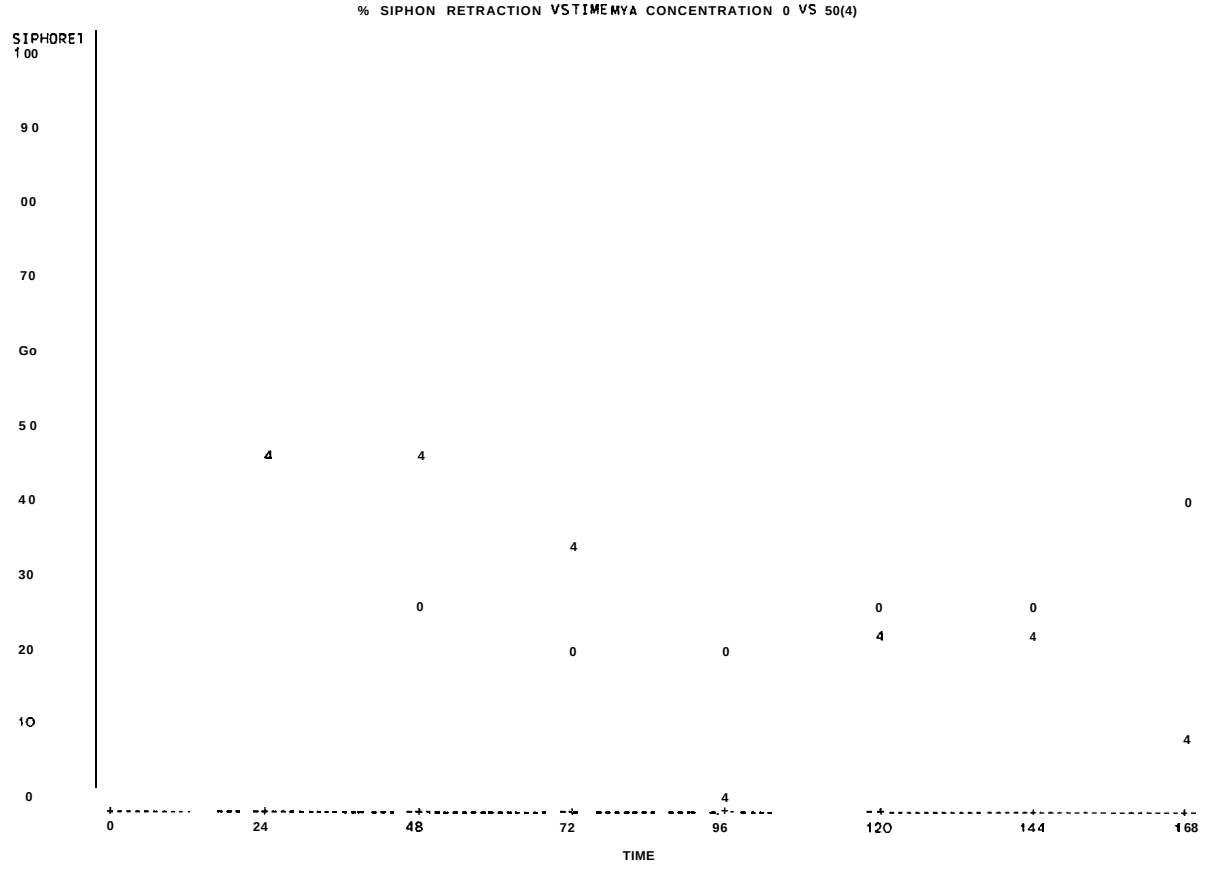


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TABLE 5 BEHAVIORAL RESPONSES OF MYA TRUNCATA EXPOSED TO CRUDE OIL DISPERSIONS IN SHORT TERM AND CHRONIC EXPOSURES AT CONSTANT LEVELS*

Exposure[a] Condition	Ostial Closure	Siphon Retraction	Stimulus Response -Siphon	Stimulus Response -Mantle
ET 50 - 1982 Statistical reduction				
Oil				
0.5	< 0.5, < 7h	NC	NC	NC
5.0	NC	< 0.5, 12h	NC	5d
50	< 0.5h	< 0.5h	NC	NC
100	0.5h	< 0.5h	NC	1d
250(cv, sml)			2d	
250(cv, lg)	2h			
500	< 6h	< 0.5h	12h	6h
50ppb(cv)	NC	NC		
500ppb(cv)	NC	NC		
COREXIT				
50	< 0.5h	4h	< 0.5h	< 0.5h
RECOVERY				
oIL				
0.5	3d	NC	NC	NC
5.0	NC	1d	NC	6d
50	2d	3d	NC	NC
100	12h	3d	NC	2d
250 (cv, sml)	1d	1d	2d	1d
250 (cv,lg)	1d	1d	5d	Id
500	4d	4d	Id	1d
50ppb(cv)	NC	NC		
500ppb(cv)	NC	NC		
COREXIT				
50	1d	3d	4h-1d	4h-1d

a 6 Hours of exposure to Lago Medio crude oil or Corexit 9527 dispersions, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as CV; animal size medium except where noted as sml or lg, all concentrations in ppm except as noted.

NC = no change from control or maximum response less than 20%

- = no data, due to inaccessibility of animals because of covered system or to inability to observe behavioral change in dispersion-clouded water

ET 50 = time from start of exposure at which one half of the starting test population showed the index response

TABLE 6 BEHAVIORAL RESPONSES OF MYA TRUNCATA EXPOSED TO CRUDE OIL DISPERSIONS IN A SEQUENCE OF THREE EXPOSURE LEVELS.

Exposure[a] Condition		Ostial Closure	Siphon Retraction	Stimulus Response -Siphon	Stimulus Response -Mantle
ET 50					
A.	0.5/5.0/0.2	NC	NC	NC	NC
B.	0.5/5.0/0.2(cv)	NC	NC		
A.	10/100/5.0	2h	6.5h	1d	13d
B.	10/100/5.0(cv)	1h	7h[b]		
RECOVERY					
A.	0.5/5/0.2	NC	NC	NC	NC
B.	0.5/5/0.2(cv)	NC	NC		
A.	10/100/5	18h	18h	5d	1d
B.	10/100/5(cv)	3d	1d		

a 18 Hours of exposure to Lago medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv.

NC = no change from control or maximum less than 20.

- = no data due to inaccessibility of animals because of covered system or to inability to observe behavioral change in dispersion-clouded water.

b Indicates that maximum response shown by less than 50% of starting population, generally 20-40%.

A 1982 Statistical reduction
B 1983 statistical reduction

ET 50 time from start of exposure at which one half of the starting test population showed the index response

At a concentration of 250 ppm, emergence from the sediment and increased gaping of the mantle opening and shell became pronounced responses. Recovery times for all behavioral indices were greatly lengthened.

At 500 ppm all behavioral indices were affected. Biphasic responses were noted for mantle/shell gape, foot protrusion, siphon and mantle reaction to mechanical stimuli. The second onset of the behavioral responses was latent, sometimes occurring after the exposure period. The time required for 50% of the population to return to control behaviour was much greater at 500 ppm than for all other concentrations. This delay further supported the dose response trend of increased recovery time with increasing concentration. Lethality was delayed at this concentration, the ET 50 occuring at 6 days. The data on the effect of differential sizes of test organisms during the 250 ppm exposure were inconclusive.

Exposure to 50 ppm of Corexit resulted in ostial closure, emergence from the sediment and mantle gaping. Time to recovery from this exposure was much shorter than the 500 or even 250 ppm exposures. No lethality was recorded during this test.

S. groenlandicus response to the prolonged 50 ppb exposure was latent and limited to ostial closure and mantle gaping during the first 10 days. The 1/2 day ET 50 for ostial closure agreed well with the 1982 observations of longer reaction times for animals exposed to lower concentrations. However at day 11 of the exposure period, mass emergence from the sediment occured. Up to 90% of Serripes groenlandicus were seen on the surface with open but not gaping shells. By day 14, 95% of the bivalves had gaping shells, protruding feet but still responded to strong stimulus. No recovery was observed or expected. Exposure to 500 ppb resulted in a biphasic ostial closure response, the first incidence occuring after 1 day of exposure and a second at day 12. A corresponding but limited emergence from the sediment occured at day 1 was followed by reburrial by day 4.

A pronounced tendency for emergence corresponded with the second ET 50 for ostial closure. The narcosis intensity in S. groenlandicus exposed to 500 ppb was manifested by a total lack of reaction to mantle or siphon stimulus, however, shell gaping, foot protrusion werenot pronounced. Recovery times for the 500 ppb chronic exposure were outside the experimental period. A comparison of the effects from the 1982 short term low dose exposure (0.5 ppm) with those resulting from the prolonged exposure to similar levels 500 ppb during 1983 indicated S. groenlandicus to be unable to adjust to chronic contamination even at low levels.

The Bay 9 and Bay 10 field spill simulations resulted in unique response patterns (Table 8). The initial 6 hour exposure to 0.5 or 10 ppm of the three phase exposure regime resulted in an enhanced response to the mid-sequence high concentration as compared to the corresponding single dose exposures of 5 and 10 ppm. Recovery times were also delayed in the sequential

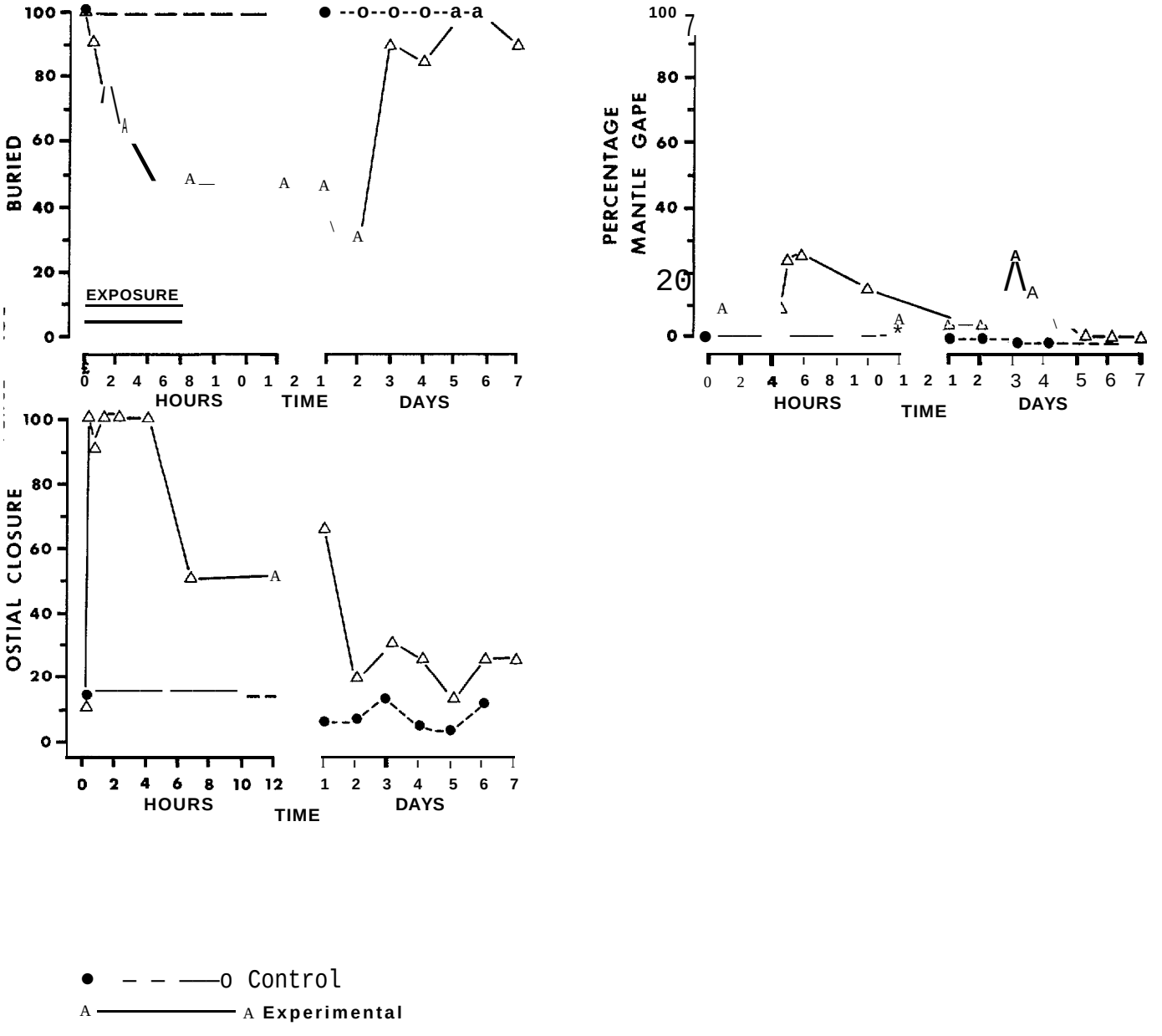
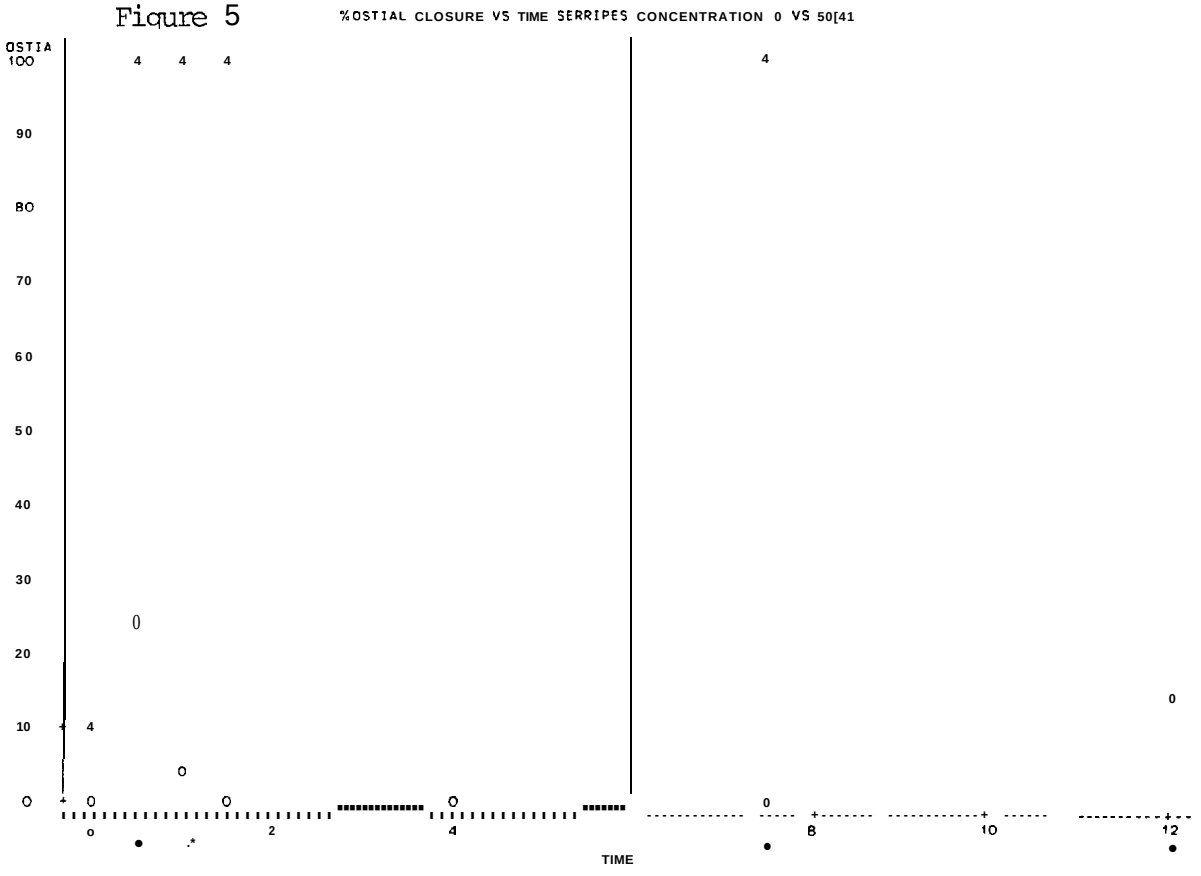


Figure 4

Selected behavioral responses of **Serripes groenlandicus** to 50 ppm dispersed oil

Figure 5



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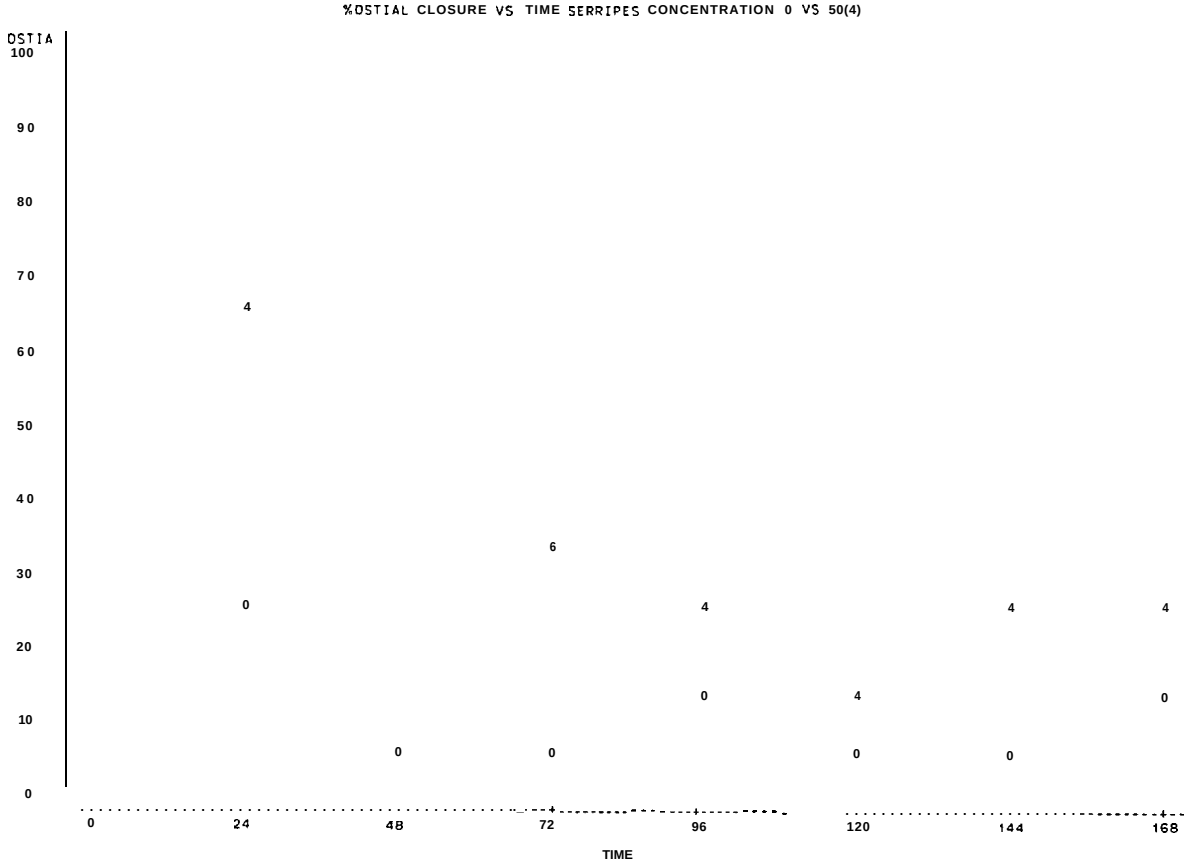

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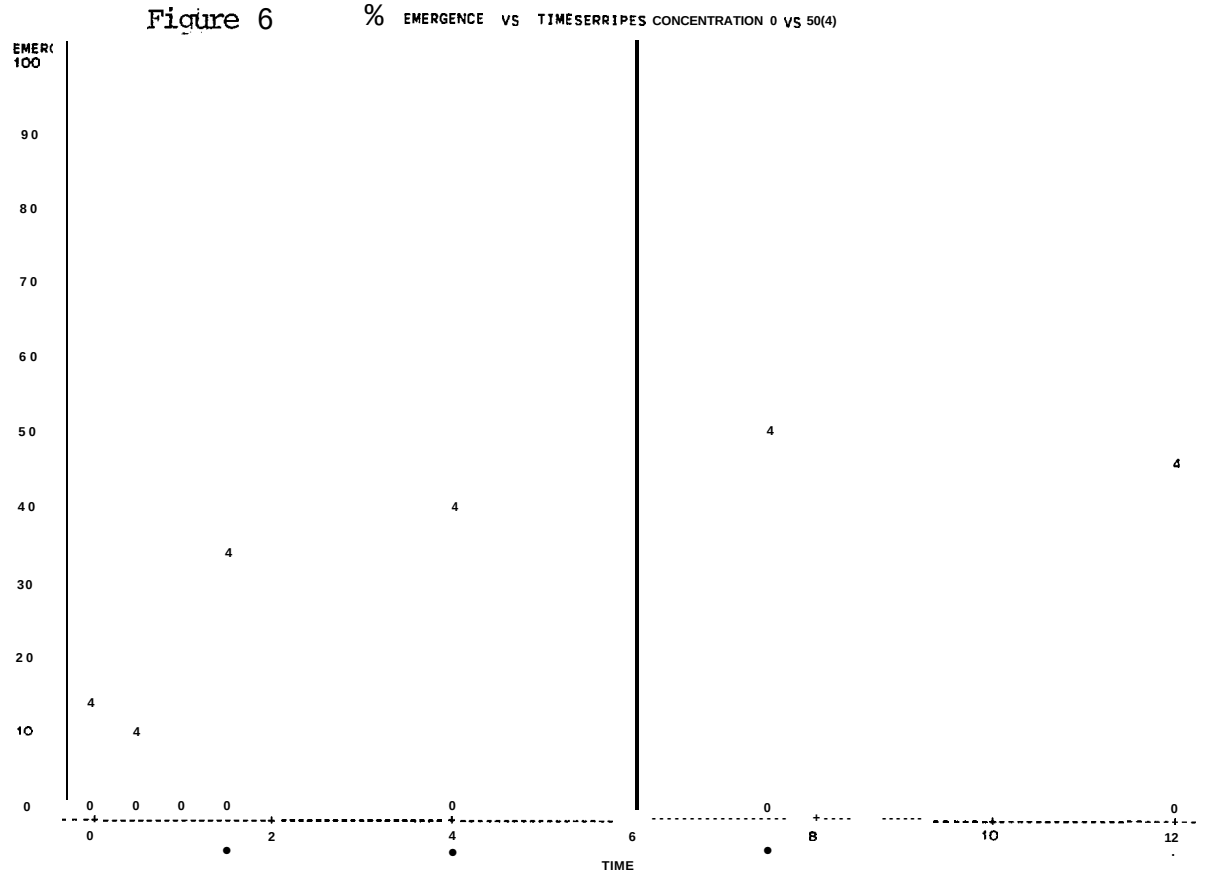
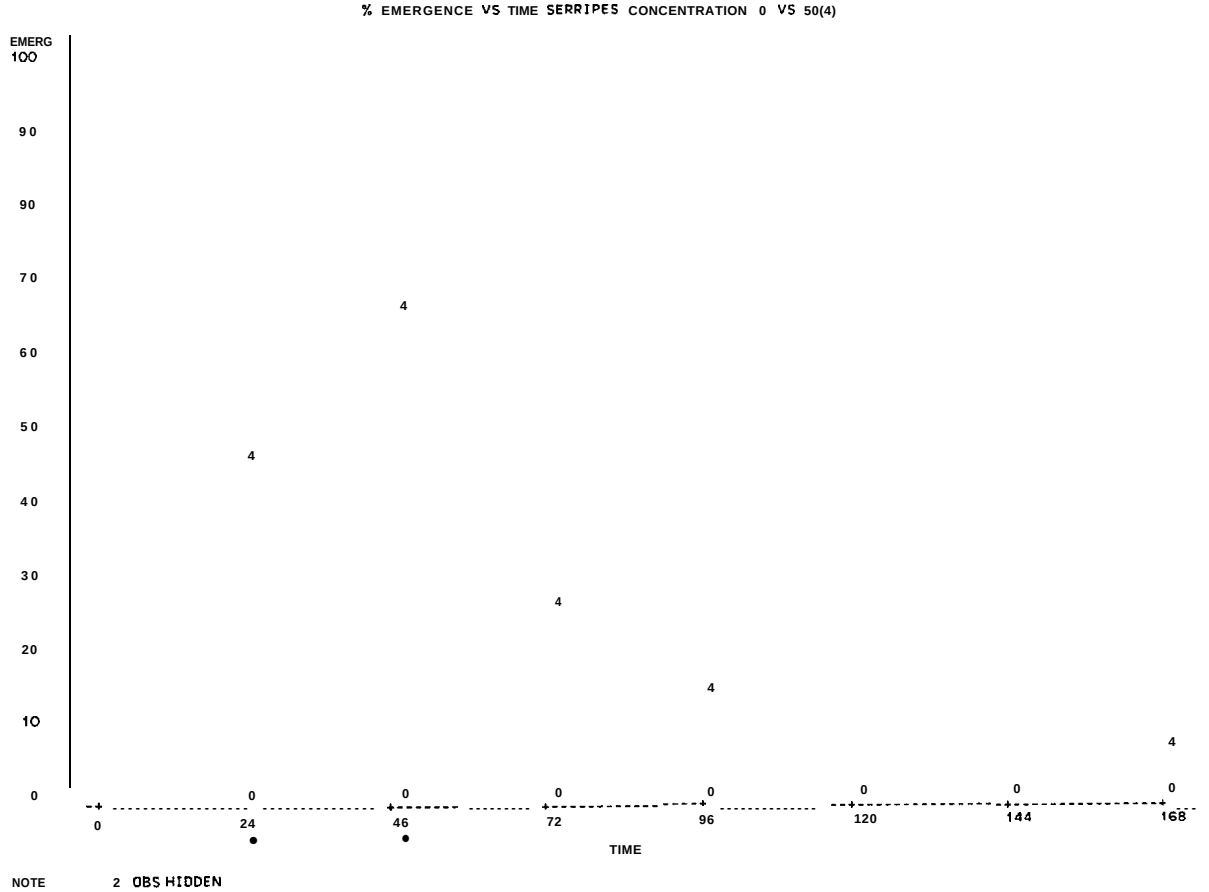
Figure 6





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exposure tests. Although the use of tank covers to minimize evaporative hydrocarbons loss did not consistently alter the responses to sequential exposures, all 1983 dosing tanks were covered to minimize the potential loss. Repetition of the sequential exposure tests in 1983 further substantiated the validity of the in situ replication of the 1981 field conditions.

An additional response was inferred from the ostial closure observations. Siphon retraction was probably an accompanying response, which could not be quantified given the normal attitude of the bivalve. This attitude was characterized by burial to various depths in the sediment with only the tip of the siphon and ostia showing. Siphon retraction and forceful closure were probable occurrences at concentrations of 50 ppm and greater, as evidenced by a high incidence of shell damage. Individuals which emerged from the sediment tended to be cracked along margins of their valves. At times this involved as much as one third of the shell surface resulting in the exposure of soft tissues. Forceful closure of the two valves was an additional latent response which occurred at high concentrations, during both the 500 ppm single dose and the 10/100/5 ppm sequential exposure. Shell damage resulted from tight opposition of the valves, pinching an extruded foot. Increased locomotion occurred when the extruded foot contacted the sediment and straightened rapidly to propel the shell. Locomotion was erratic and observed primarily in emerged individuals at the beginning of exposure to single concentrations of 250 and 500 ppm, and to the serial 10/100/5 ppm exposure. A similar style of locomotion was observed only rarely during the pre-exposure period.

The test to determine the behavioral response of S. groenlandicus to 30 ppm oiled sediment was inconclusive. Neither ostial closure nor emergence response were consistent throughout the replicate tanks. Hydrocarbon uptake did occur however.

Stongylocentrotus droebachiensis

This test organism appeared most sensitive to oil and displayed a broad range of behavioral indices affected by oil. The initial response of S. droebachiensis to oil exposure was characterized by the-loss of covering material as the aboral tube feet released pieces of seaweed (Fucus sp) and shell fragments (table 9, Figures 7-14). Loss of covering showed a dose response beginning within 4 hours of dosing at 10 ppm (Table 10) and much earlier at high concentrations (Table 9). Loss of another tube foot function at low concentrations further documented the sensitivity of S. droebachiensis to

TABLE 7 BEHAVIOURAL RESPONSES OF SERRIPES GROENLANDICUS EXPOSED TO CRUDE OIL DISPERSIONS IN SHORT TERM AND CHRONIC EXPOSURES AT CONSTANT LEVELS*

Exposure[a] Condition	Emergence	Ostial Closure	Mantle Gape	Foot Protrusion	Stimulus Response - Siphon	Letality
ET 50 - 1982 Statistical reduction						
OIL						
0.5	NC	12h	NC	NC	NC	NC
5.0	NC	< 0.5h	NC	NC	NC	NC
50	6h	< 0.5h	NC	NC	NC	NC
100	> 6h	< 0.5h	NC	NC	NC	NC
250 (CV sml)	6h	< 5h	1d[b]	NC		NC
250 (cv lg)	6h	< 6h	3d[b]	NC		NC
500	6h	< 6	< 0.5h,3d	< 0.5h,1d	< 0.5h,7d	6d
50ppb (CV)	1 1d	1/2d,11d	14d			NC
500ppb (cv)	1d[b] 1 1d	1d, 12d				NC
COREXIT						
50	4h 4h	2h 1/2h	4h	NC		NC
RECOVERY						
OIL						
0.5	NC	1d	NC	NC	NC	NC
5.0	NC	2d	NC	NC	NC	
50	3d	4d	NC	NC	NC	NC
100	2d	2d	NC	NC	NC	NC
250 (CV sml)	5d	5d	5d	NC		NC
250 (cv lg)	3d	6d	5d	NC		NC
500	> 7d	> 7d	1d,7d	2h,7d	3d	
50 ppb (cv)	4d[b],>14d	2d, >14d				
500 ppb (cv)	>14d	>14d				
COREXIT						
50	2d	2d	NC	NC		NC

ET 50 = time from start of exposure at which one half of the starting test population showed the index response

a 6 Hours of exposure to Lago Medio crude oil or Corexit 9527 dispersions, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as CV; animal size medium except where noted as sml or lg, all concentrations in ppm except as noted.

b Indicates the maximum response shown by less than 50% of starting test population, generally 20-40%

NC = no changes. from control or maximum response less than 20%

= no data, due to inaccessibility of animals because of covered system or to inability to observe behavioural change in dispersion-clouded water

TABLE 8 BEHAVIORAL RESPONSES OF SERRIPES GROENLANDICUS EXPOSED TO CRUDE OIL DISPERSIONS IN A SEQUENCE OF THREE EXPOSURE LEVELS.

Exposure[a] Condition	Emergence	Ostial Closure	Mantle Gape	Foot Protrusion	Response - Siphon	Lethality
ET 50 A. 1982 Statistical reduction B. 1983 Statistical reduction						
A. 0.5/5/0.2	15	6h	NC	NC	NC	NC
B. 0.5/5/0.2(cv)	13h	8.5h				
A. 10/100/5	14h	< 1/2h	7h, 2d	>0.5h<10h,1d	7h	NC
B. 10/100/5 (cv)	15h	< 1/2h				
RECOVERY						
A. 0.5/5/0.2	2d	2d	NC	NC	NC	NC
B. 0.5/5/0.2(cv)	1.5d	4d				
A. 10/100/5	7d	5d	≥ 3h,≤13h,7d	≥2h≤13h,2d	3d	NC
B. 10/100/5(cv)	7d	2d				

[a] 18 Hours of exposure to Lago Medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as CV, all concentrations in ppm.

NC = no change from control or maximum response less than 20%

– = no data due to inaccessibility of animals because of covered system or to inability to observe behavioral change in dispersion-clouded water.

ET 50 = time from start of exposure at which one half of the starting test population showed the index response.

oil. The urchins' inability to remain attached to the walls of the aquarium through the use of their tube feet from 5 ppm on and the increase in onset of this response with higher concentration indicated a dose related response. Recovery times for these tube feet functions ranged from 3 to 5 days except for animals exposed to 500 ppm. Although urchins exposed to 500 ppm regained the ability to decorate themselves within 30 days, there was no recovery of attachment ability within the 7 day experimental period.

Curling and retraction of aboral tube feet occurred at most concentrations (Table 9, 10). Curling and retraction occurred in but a few of the animals exposed to the chronic 50 ppb dose. At 250 ppm, curling of the ostial ends of the tube feet was bi-phasic. Retraction and curling of tube feet were dose response reactions, occuring more rapidly at high doses and requiring longer time for recovery of normal attitudes. Although the onset of retraction of tube feet was rapid at high doses, not all tube feet retracted simultaneously. A small number of oral and aboral tube feet often continued to attach the urchin to the glass surface.

Changes in spine behaviour were observed in animals exposed to concentrations of 5 ppm and more. These responses occurred more rapidly and recovered more slowly with increasing concentration. Drooping of aboral spines occurred in two phases, during both exposure and recovery periods for concentrations of 250 ppm and more. The "pincushion" effect characterizing spine rigidity tended to be a rapid response. Spine attitude recovery was in general more rapid than tube foot recovery.

Exposures to concentration of 5 ppm and greater resulted in a loss of response capacity to mechanical stimuli, with ET50 times decreasing from 5 h to less than 1 h (Table 9). Recovery times were not well correlated with exposure concentration; 1 to 5 days elapsed before urchins regained normal stimulus reactions. Spinal responses recovered more rapidly than tube foot responses. A lethal effect was recorded only when spine and tube foot responses to tactile stimulation were unobtainable. A 3 day ET50 for lethality was recorded for the 500 ppm single exposure.

Additional behavioral responses of S. droebachiensis exposed to oil were recorded. During the first few minutes following the addition of despersed oil to the 50, 100 and 250 ppm exposure, the urchins appeared to increase their movement along the substrate surface. Darkening of the test colour was also a common response to high level exposures. Shedding of gametes occurred frequently within two days of exposure. In the days preceding death, spines tended to drop from the dying urchins.

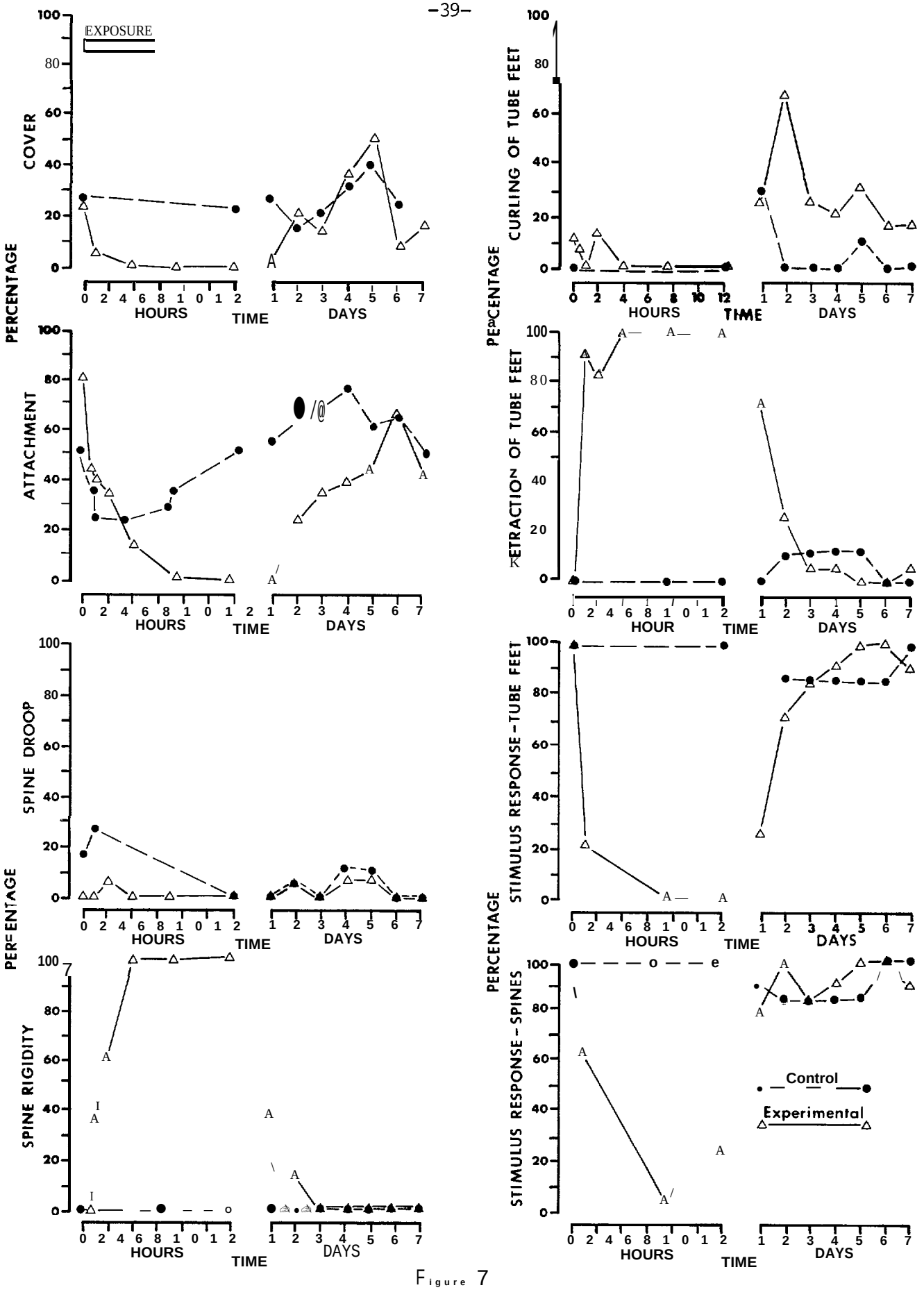
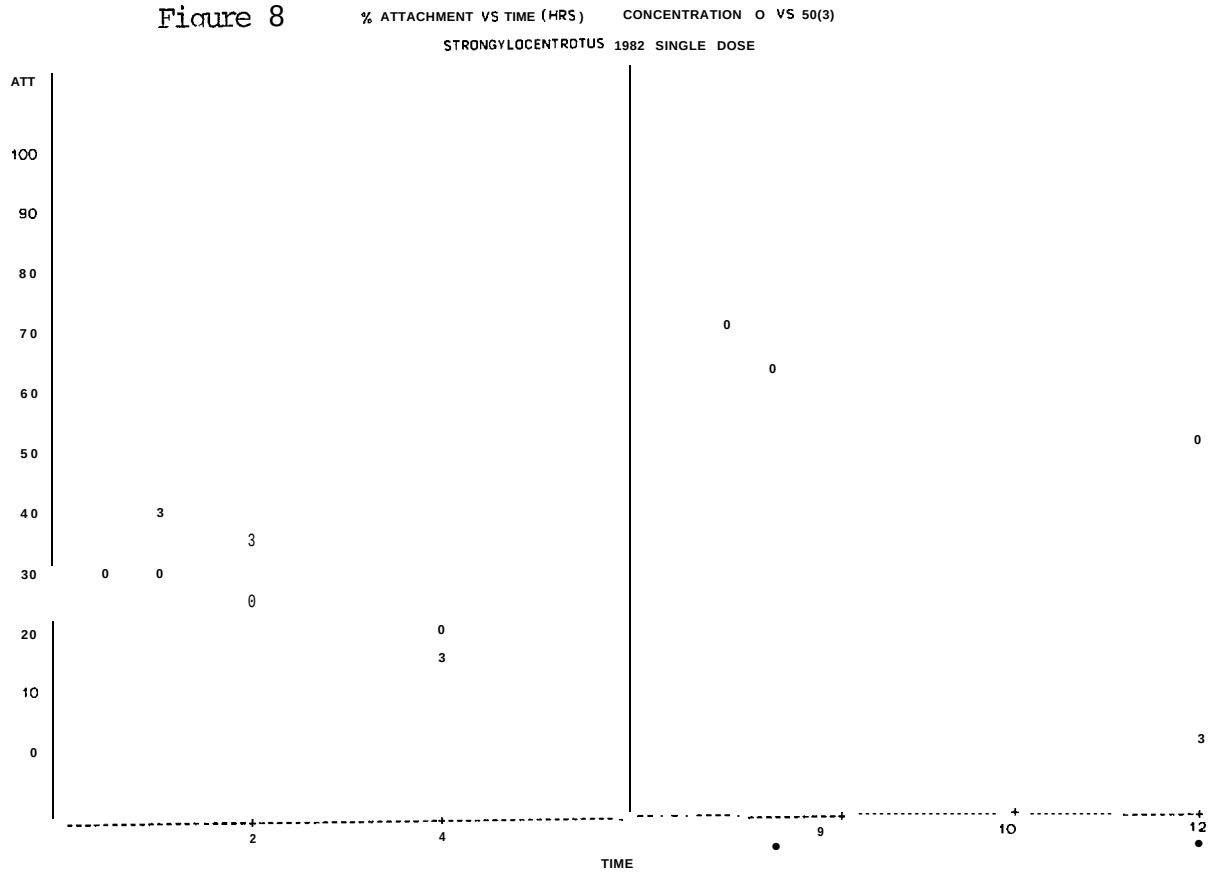


Figure 7

Selected behavioral response of Strongylocentrotus droebachiensis to 50 ppm dispersed oil

Figure 8



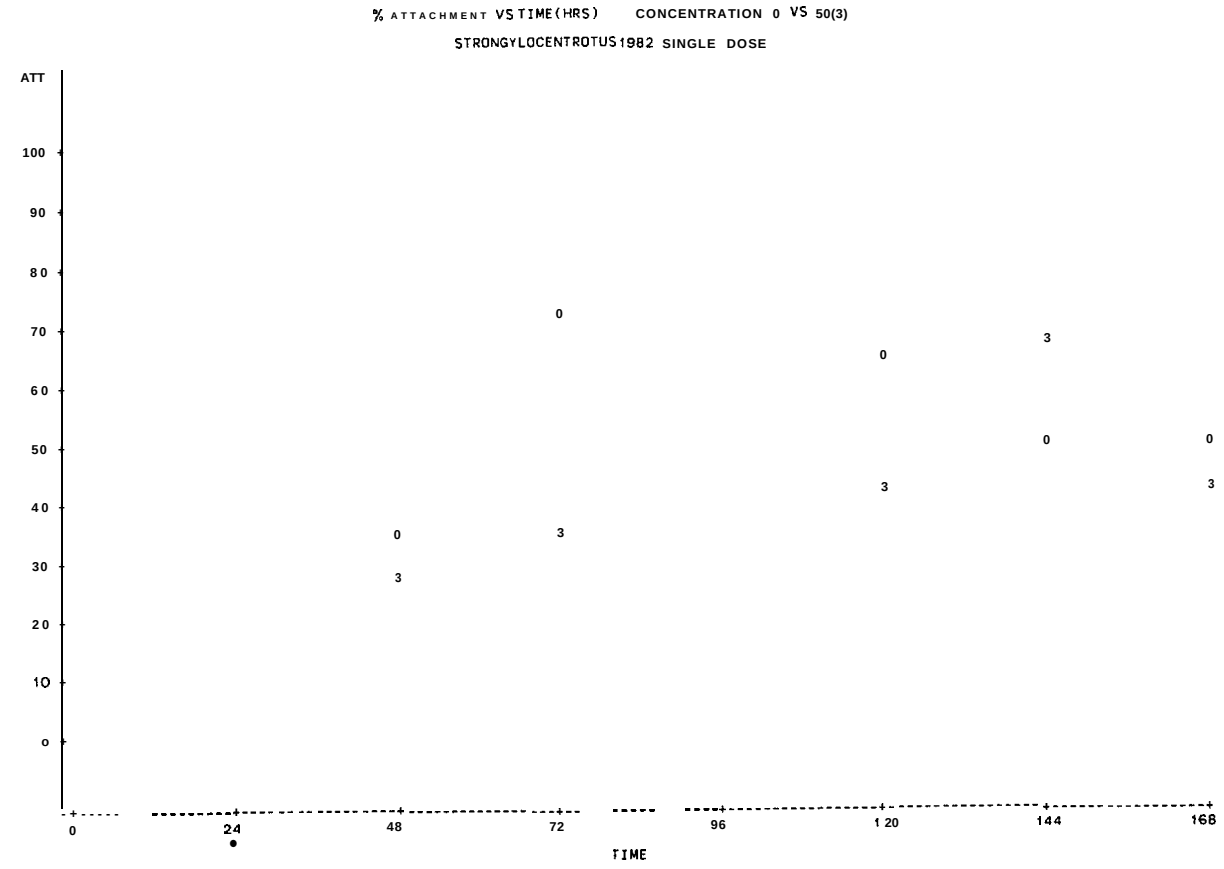


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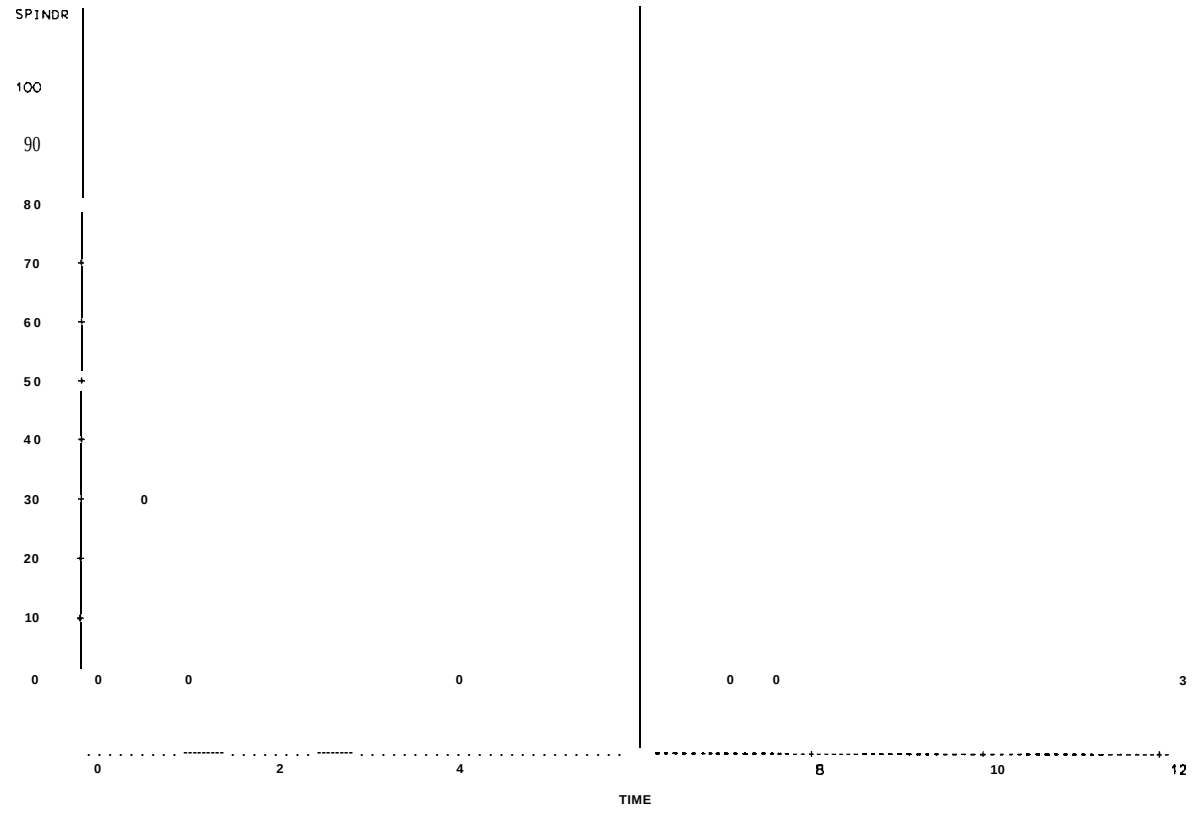
Figure 9 % SPINE DROOP VS TIME(HRS) CONCENTRATION 0 VS 50(3)

STRONGYLOCENTROTUS 1982 SINGLE DOSE



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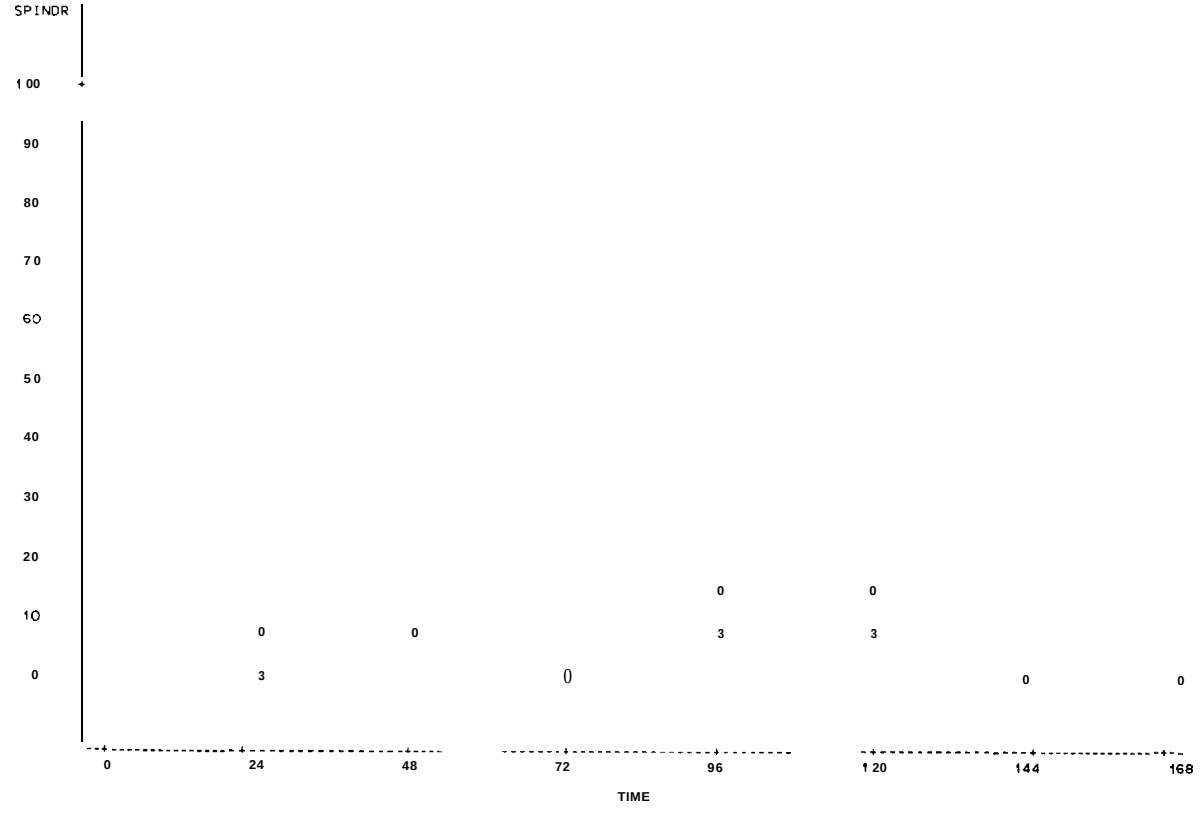
% SPINE DROOP VS TIM E(HRS) CONCENTRATION 0 VS 50(3)

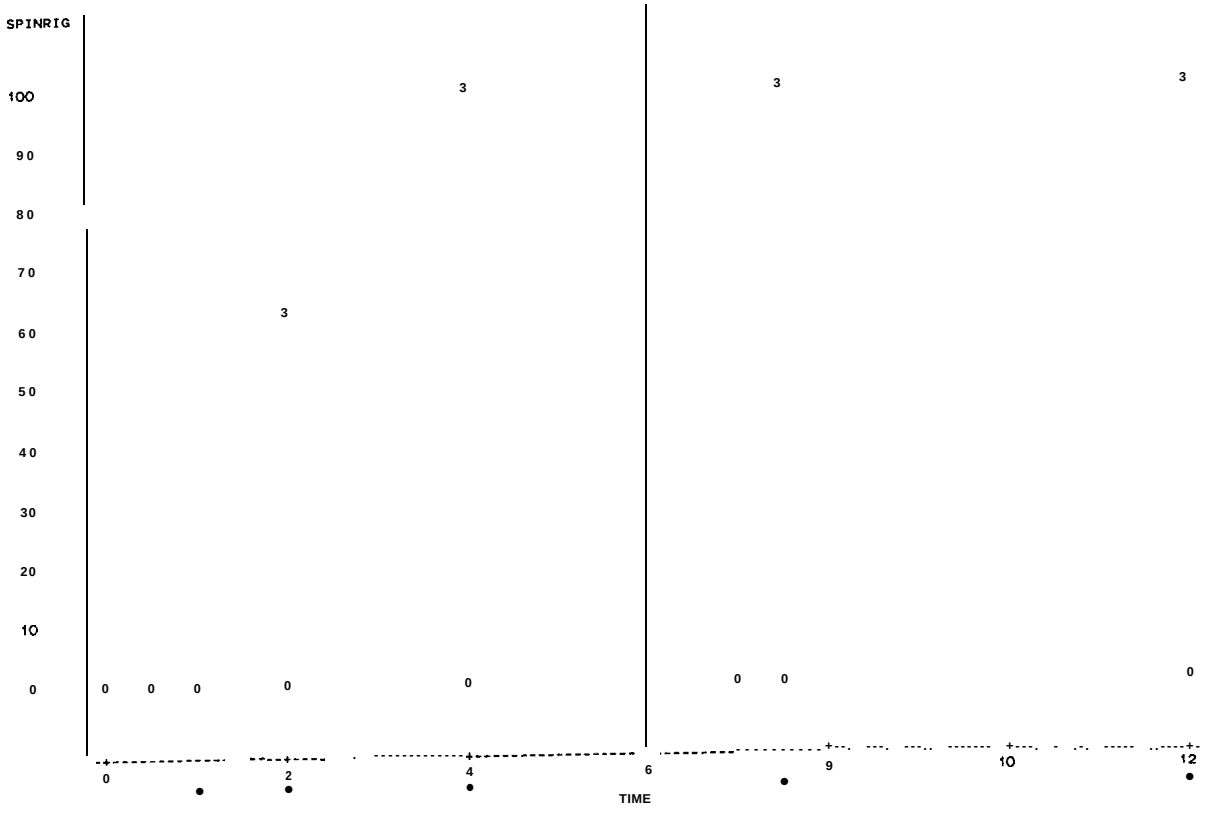
STRONGYLOCENTROTUS 1982 SINGLE DOSE



NOTE 4 OBS HIDDEN

"Significantly different values ($p < 0.05$)
Missing obs. or obs. out of range = missing value in one of the comparisons at that time
Hidden observations = two values occupy the same coordinates on the graph, only one value is printed

Figure 10 % SPINE RIGIDITY VS TIME(HRS) CONCENTRATION O VS 50[3]
STRONGYLOCENTROTUS 1982 SINGLE DOSE



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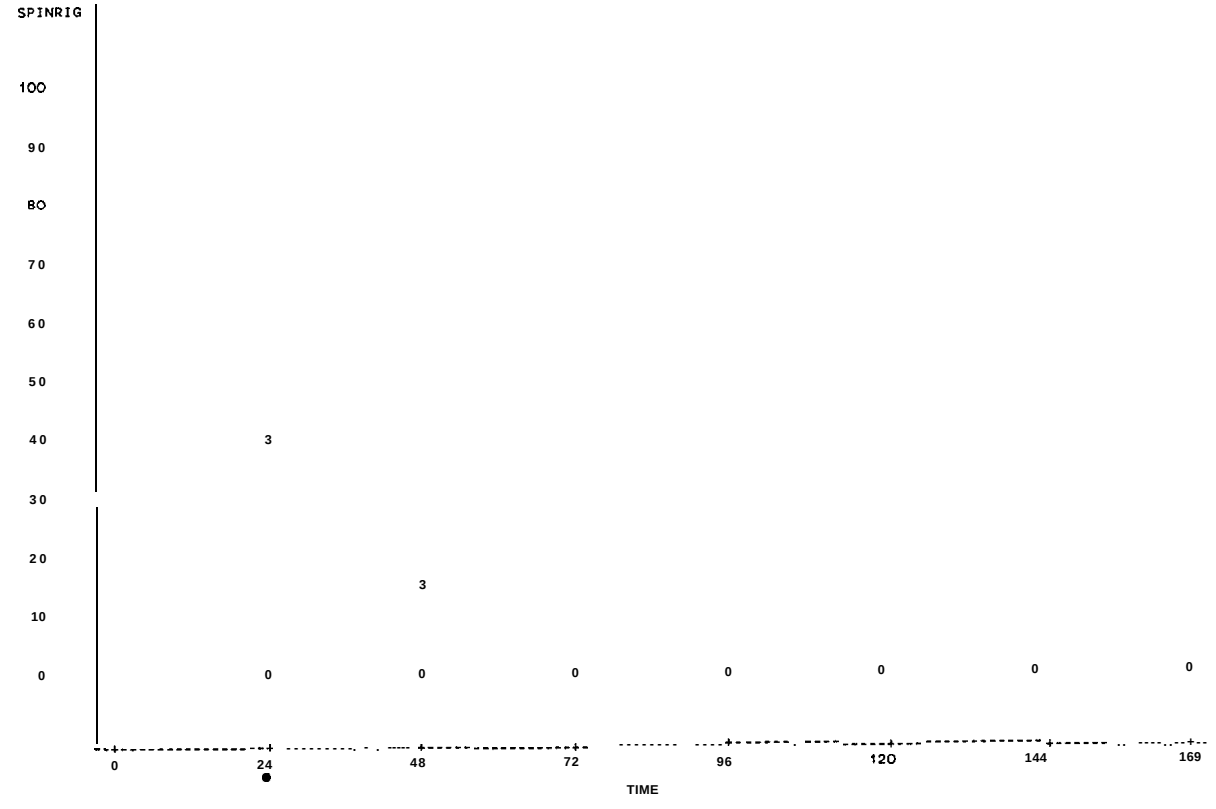
% SPINE RIGIDITY VS TIME(HRS) CONCENTRATION 0 VS 50(3)
STRONGYLOCENTROTUS 1982 SINGLE DOSE



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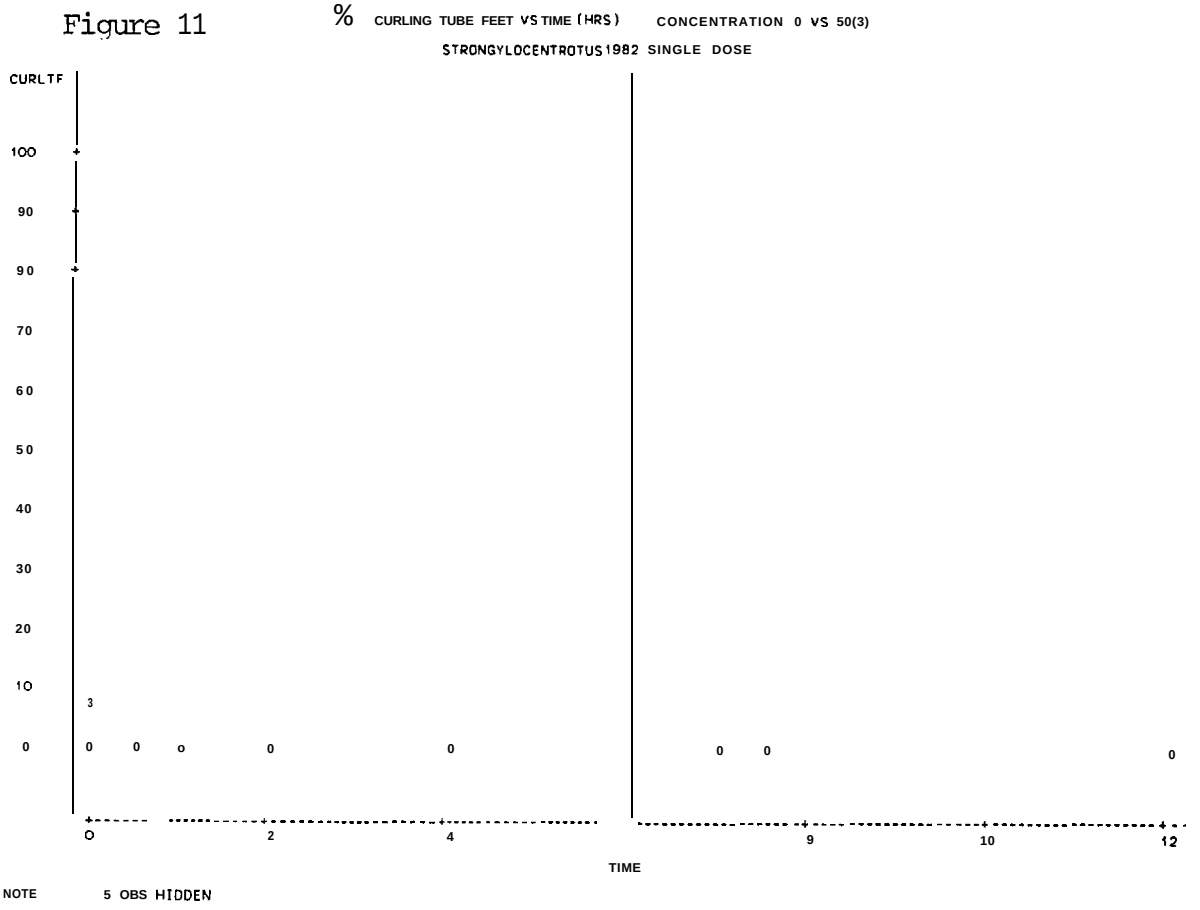
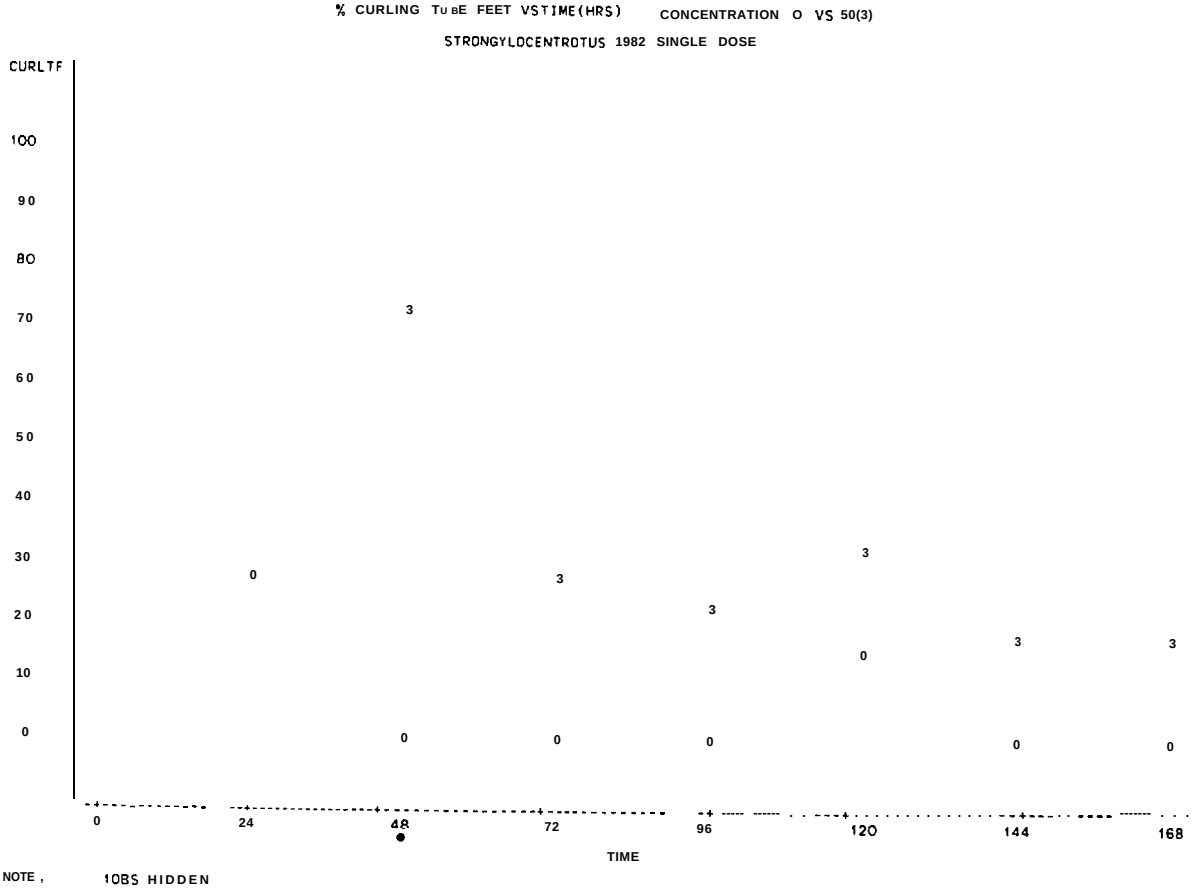
idden observations = two values occupy the same coordinates on the graph, only one value is printed

Figure 11





"Significantly different values ($p < 0.05$)

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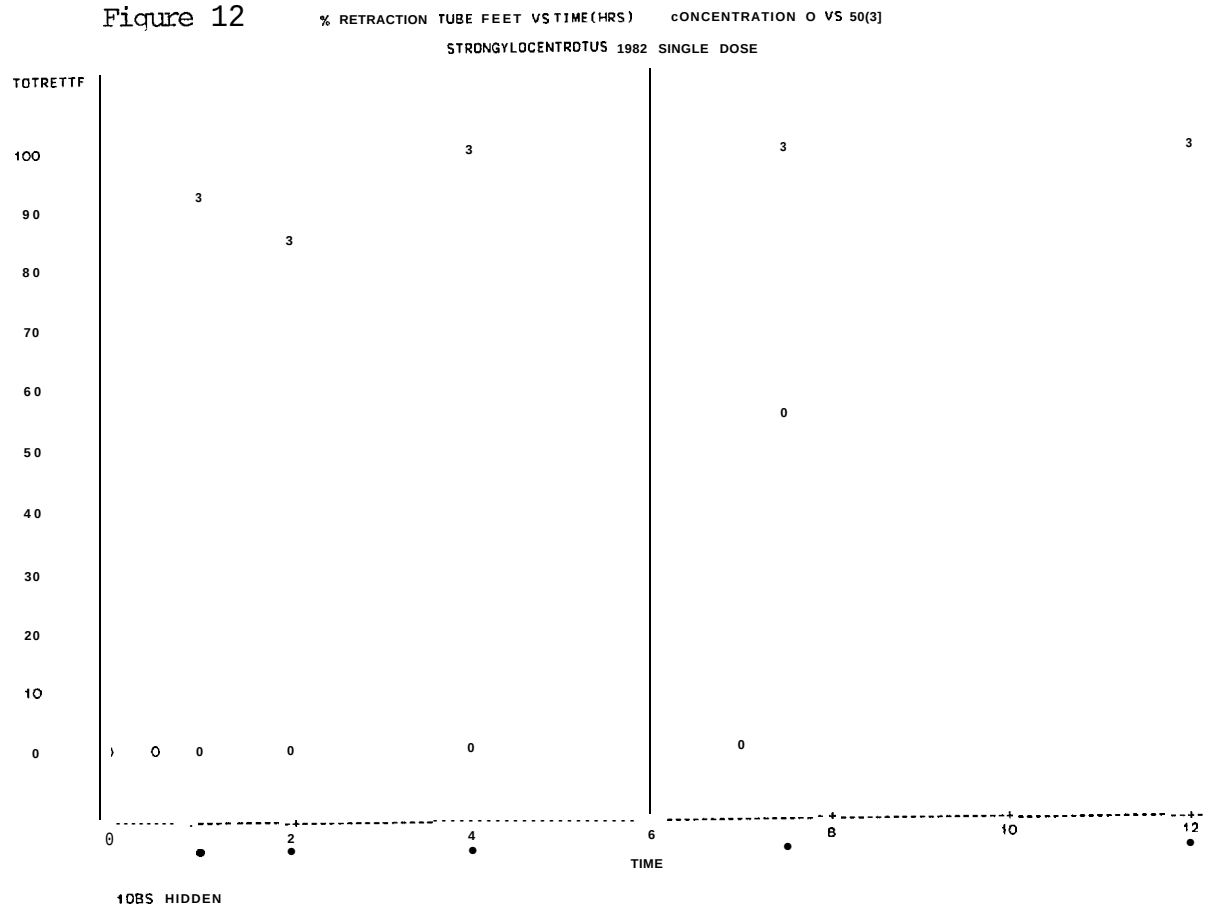
Figure 12



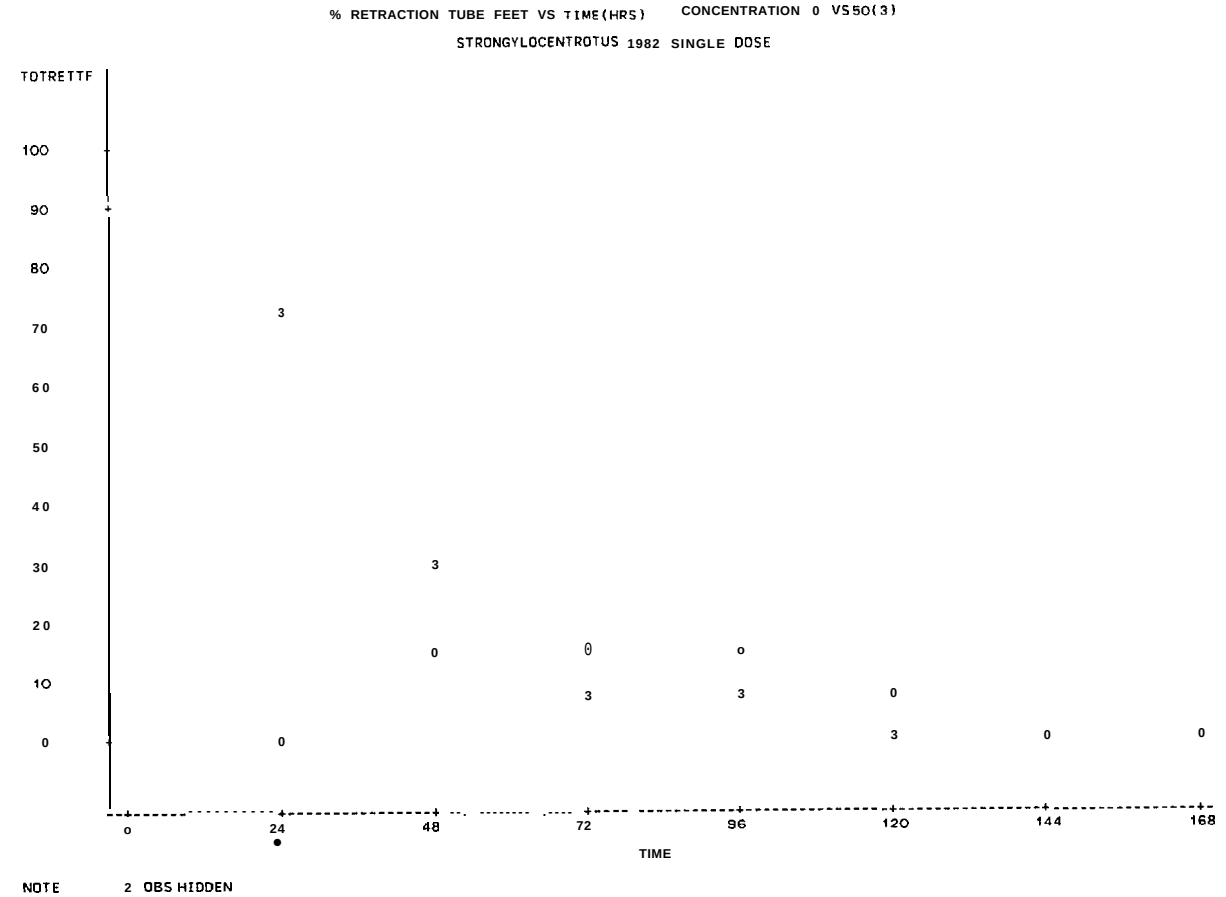


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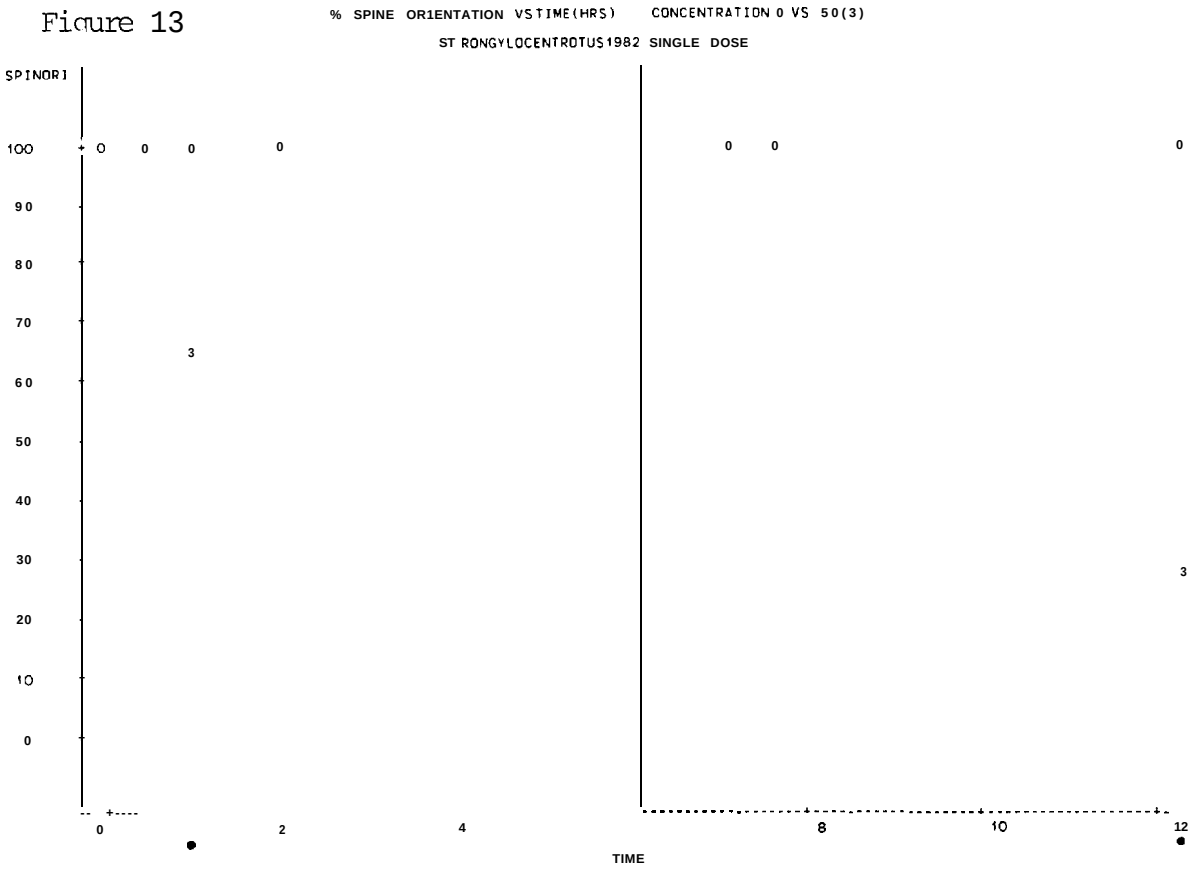
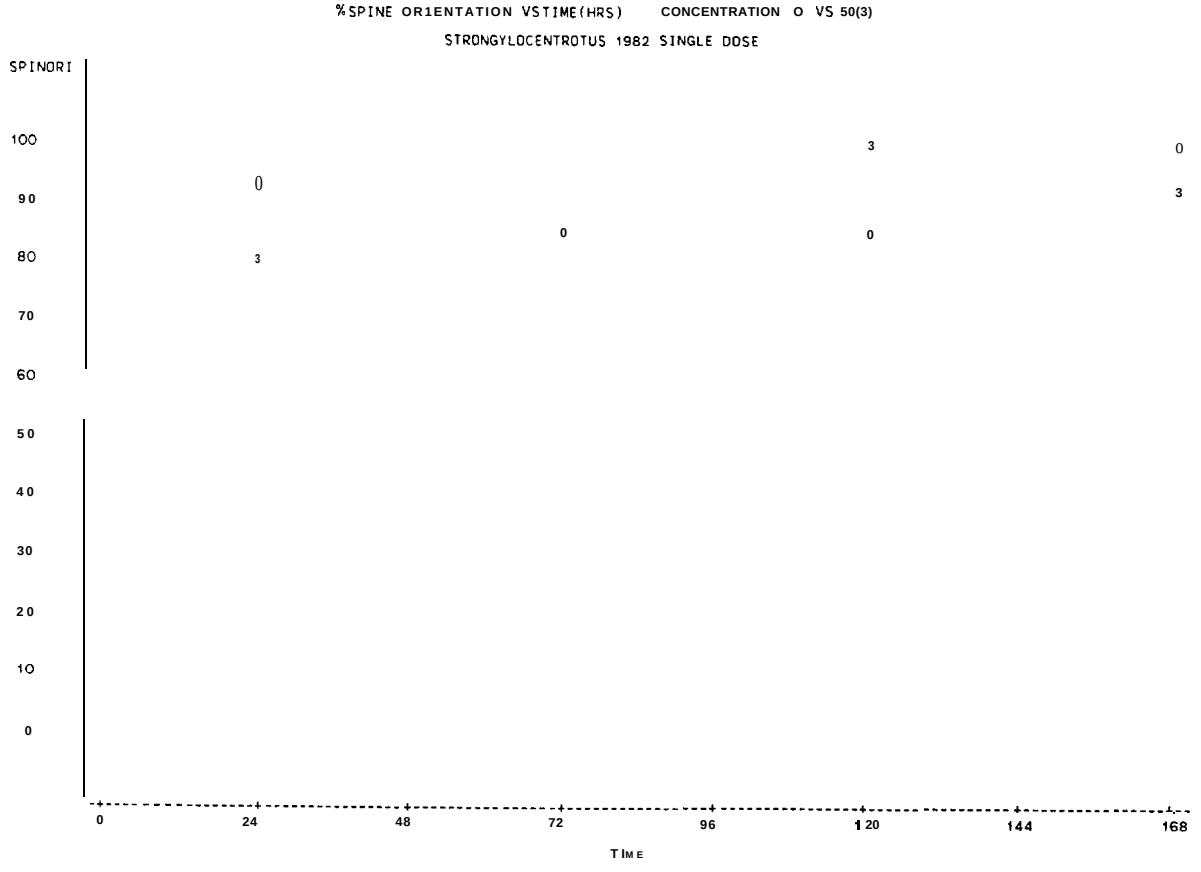
Figure 13



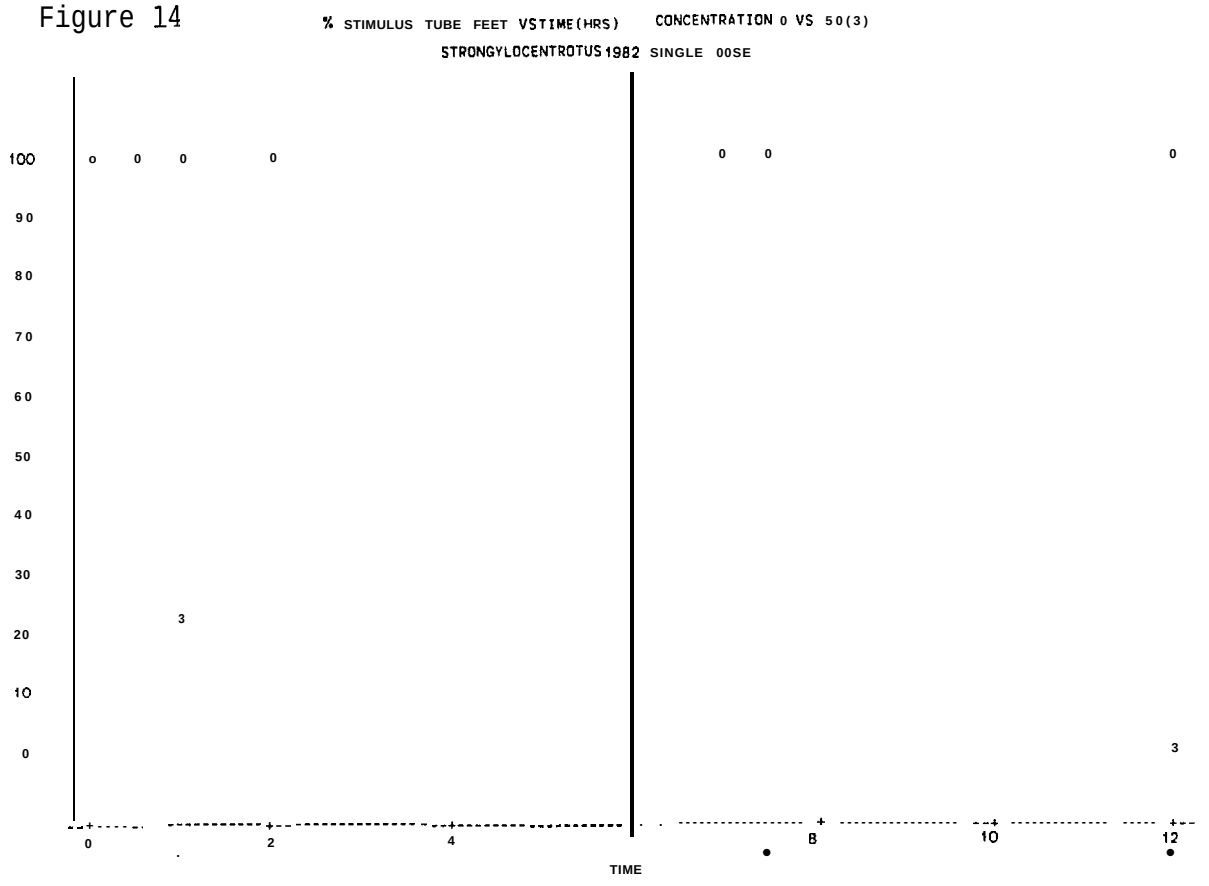
NOTE 4 OBS HAD MISSING VALUES OR WERE OUT OF RANGE



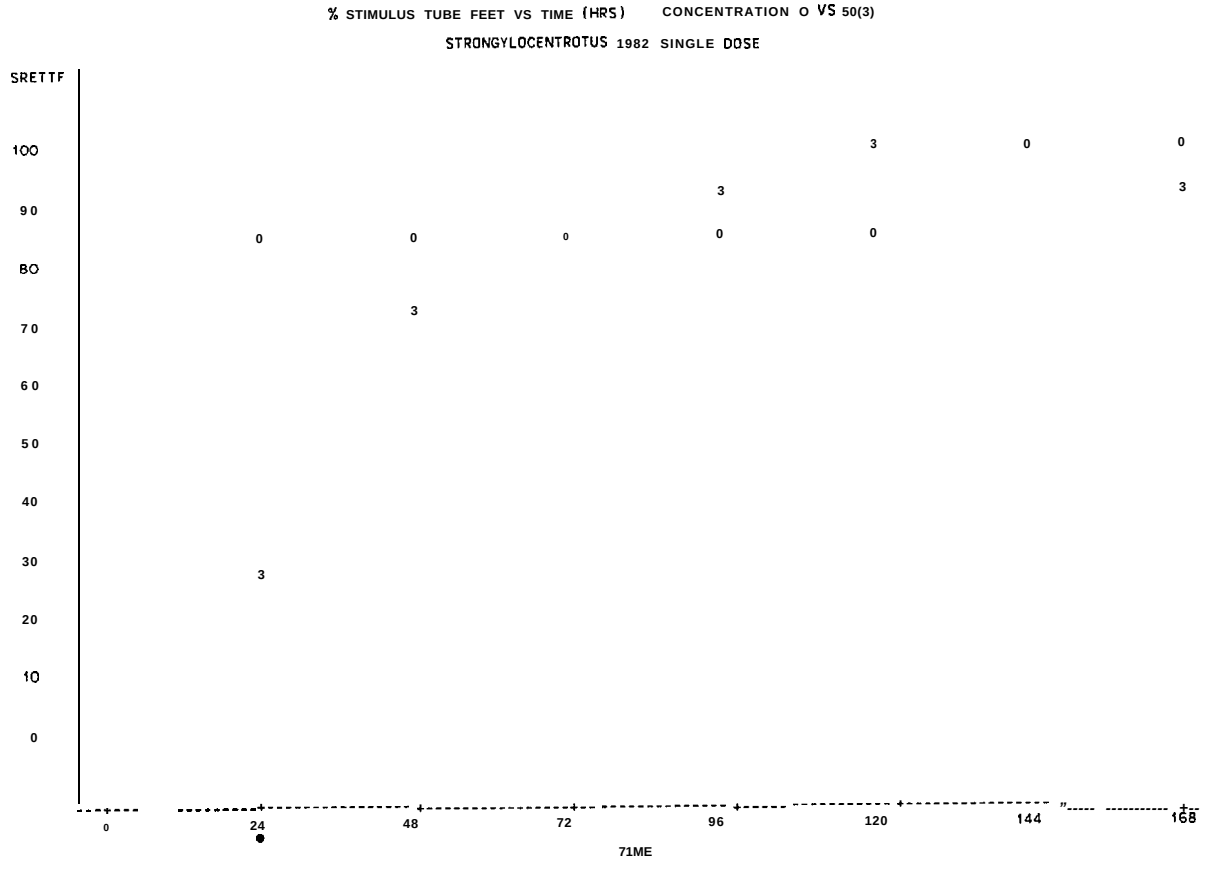
NOTE 2 OBS HIDDEN

Significantly different values ($p < 0.05$)
Missing Obs . or obs. out of range . missing value in one of the comparisons at that time
Hidden observations = two values occupy the same coordinates on the graph, only one value is printed

Figure 14



NoTE 3 OBS HAD MISSING VALUES OR WERE OUT OF RANGE



NOTE: 2 OBS HIDDEN

ıificantly different values ($p \leq 0.05$)
ɜing obs. or ohs. out of range = missing value in one of the comparisons at that time
len observations = two values occupy the same coordinates on the graph, only one value is printed

No size effects were found in urchins. Exposure to 50 ppm of Corexit resulted in loss of adhesion and spine rigidity, but recovery of both functions was much more rapid than for any dispersed oil exposure.

Spine droop, tube foot curling and retraction occurred in animals exposed both to the single 0.5 ppm concentration and in those exposed to the chronic 500 ppb dose. The onset of these behavioral indices was substantially delayed in the chronic test as was the recovery of normal attitudes of spines and tube feet (Table 9).

The data shown on Table 10 suggest that 6 h of exposure of 0.5 ppm enhanced the effect of 5 ppm given in a subsequent dose, as compared to a 5 ppm single exposure. Since 10 ppm exposures were effective in eliciting all behavioral changes within a 6 hour time period, it was not possible to differentiate the behavioral responses of 100 ppm single and sequential exposure. A comparison test between covered and non-covered systems showed that the urchin response and recovery were similar.

1983 data however indicate a change in the overall sensitivity of S. droebachiensis to oil exposure. Although dosing conditions in 1983 were identical to those described for 1982, behavioral indices, their onset and recovery time differed (Table 10). Notable differences in behaviour of urchins exposed to the Bay 9 sequential dosing in 1983 included a delayed although still biphasic occurrence of spine droop, an early onset but delayed recovery of loss of attachment ability, and a total absence of spine rigidity. Delayed tube foot curling also seemed to indicate a more oil resistant animal in 1983. Another difference between 1982 and 1983 urchin populations was absence of gamete release common during the experimental period of 1982, and the non-gravid condition of the gonads observed during dissection of the 1983 samples. Sectioned 1982 animals were characterized by enlarged and brightly coloured gonads indicative of a sexually mature population while 1983 urchins appeared non reproductive.

'ABLE 9 BEHAVIOURAL RESPONSES OF STRONGYLOCENTROTUS DROEBACHIENSIS EXPOSED TO CRUDE OIL DISPERSIONS IN SHORT TERM AND CHRONIC EXPOSURES AT CONSTANT LEVELS

Exposure[a] Condition	Loss of Cover	Loss of Attachment Ability	Spine Droop	Spine Rigidity	Curling of Tube Feet	Retraction of Tube Feet	Stimulus Response -Tube Feet	Stimulus Response - Spine	Lethality
ET 50 statistical reduction									
OIL -									
1.5	NC	NC	8h[b]	NC	1h	7h[b]	NC	NC	NC
5.0		< 2h[b]	4h[b]	< 7h	<6h	4h	<6h	< 6h	NC
50	< 1h	< 4h	NC	2h	2d	< 1h	< 1h	2h	NC
100	2h	5h	1h[b]	< 7h	2d	1h	1h	2h	NC
250	< 0.5h	<0.5h	0.5h,1d	< 0.5h	0.5h,2d	1h			NC
250 (Cv)	< 0.5h	1h	0.5h,1d	< 0.5h	2d	< 0.5h			NC
250 (cv. sml)	1h	1h	< 0.5h	0.5h	< 0.5h,3d	< 0.5h			NC
250 (cv,lg)	1h[b]	1h	< 0.5h,1d	0.5h	< 0.5h,3d	< 0.5h			NC
500	1h[b]	1h[b],2d	2h	3d	<6h	<1h	<1h		3d
50ppb (cv)		NC	NC	NC	NC				
500ppb (cv)		NC	2d	2d	<1d				
Corexit									
50	NC	0.5h[b]	< 0.5h[b]	<0.5h[b]	NC	NC	NC	NC	NC
RECOVERY OIL									
1.5	NC	NC	1d	NC	10h	NC	NC	NC	NC
5	3d	NC	NC	12h	1d	1d	4d	3d	NC
50	4d	4d	NC	2d	3d	2d	1d	3d	NC
100	4d	3d	7h	2d	3d	1d	2d	2d	NC
250	3d	> 4d	4d	1d	4d	2d	2d	1d	NC
250 (cv)	3d	4d	4d	2d	7d	3d	3d	1d	NC
250 (cv, sml)	3 d	5d	1h	3d	1h,5d	3d	3d	1d	NC
250 (cv, lg)	3d	4d	2h,3d	1d	1h,3d	2d	1d	1d	NC
500	3d	Nil	7d	1d	77 d	3d	> 5d	> 4d	NIL
50ppb (cv)	NC	NC	NC	NC	NC	NC			
500ppb (cv)	NC	NC	3d	NC	> 14d	3d			
Corexit									
50	NC	12h	2h	2h	NC	NC	NC	NC	NC

a) Six hours of exposure to Lago Medio crude oil or Corexit 9527 dispersions, followed by aeven days of post - exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv; animal size medium except where noted as sml or lg, all concentrations in ppm except as noted.

NC no change from control or maximum reponse less than 20%
= no data, due to inaccessibility or animals because of covered system or to inability to observe behavioral change in dispersion-clouded water.

) indicates that maximum response shown by less than 50% of starting test population, generally 20-408.

ET 50 = time from start of exposure at which one half of the starting test population showed the index response.

TABLE 10 BEHAVIORAL RESPONSES OF STRONGYLOCENTROTUS DROEBACHIENSIS EXPOSED TO CRUDE OIL I DISPERSIONS IN A SEQUENCE OF THREE EXPOSURE LEVELS

Exposure[a] Condition	Loss of Cover	Loss of Attachment Ability	Spine Droop	Spine Rigidity	Curling of Tube Feet	Retraction of Tube Feet	Stimulus Response -Tube Feet	Stimulus Response Spines	Lethality
ET 50									
A. 0.5/5/0.2	NC	9h	7h	<4h	2h	13h	7h	7h	NC
B. 0.5/5/ 0.2 (cv)	-	7h	9h[b]		NC	11h			
A. 10/100/5	6h	<0.5h	1h	0.5h	1h	3h	2h		
B. 10/100/5 (cv)	1.5h	NC	11d		<2h			2h	NC
RECOVERY									
A. 0.5/5/0.2 (cv)	4 d	11h	15h	15	>1d	18h	16h	13h	NC
0.5/5/0.2 (Cv)	3d[b]	14h		Nc	>14d				
A. 10/100/5 (cv)	)7 d	>7d	5d	>5d	>6d	6d	6d	>2d<6d	NC
10/100/5 (cv)	10d	NC	>14d	NC	14d				

18 hours of exposure to Lago Medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv, all concentrations in ppm.

Indicates that maximum response shown by less than 50% of starting population, generally 20 - 40 %

NC = no change from control or maximum response less than 20%

- = no data, due to inaccessibility of animals because of covered system or to unability to observe behavioral cha dispersion-clouded water.

A. 1982 Statistical reduction

B. 1983 Statistical reduction

ET 50 = time from start of the exposure at which one half of the starting test population showed the index response.

Tissue Hydrocarbons

The results of the 1982 tissue hydrocarbon determinations are presented in Table 11. Total aromatic concentrations averaged one-third of the total hydrocarbon levels. Total hydrocarbon values were mathematically derived from the sum of alkyl benzenes and naphthalenes, measured from the gas chromatograms, connected by an aromatic ratio of 0.0056. Total oil values were derived from a total aromatic to total oil ratio of 0.37.

M. truncata and S. groenlandicus tissues had a greater hydrocarbon load than did S. droebachiensis tissues when the samples were taken at 6 h of a single exposure, or 18 h of the sequential test. This relationship was reversed for the 7 d samples in which urchin tissue loads were not only greater than those of the bivalves, but also much higher than they had been at 6 or 18 h. Urchin tissues additionally showed a greater capacity to take up alkyl benzenes during the dosing period, although the emphasis on these hydrocarbons tended to be gone in 7 d samples. An overview of tissue hydrocarbons demonstrated fractional compositions qualitatively similar to that of Lago Medio crude oil.

The 1983 analytical results for total hydrocarbons optained by U.V. fluorescence and by gas chromatography are presented in Table 12. The data are presented on a wet weight basis. Conversion to dry-weight can be achieved by multiplying by a factor of 7 (Boehm et al . . 1982b).

Cross-calculation of UV/F and GC results quantifying "total petroleum" were not performed as part of the 1983 program. However, comparison of results in Table 12 indicates the UV/F and GC data "fit" together quite well.

The greatest oil uptake was observed for both M. truncata and S. groenlandicus in the Bay 9 field simulation experiment where the animals acquired 66 and 60 ppm, respectively after 6 hours. Thereafter, levels in M. truncata decreased to about 10 ppm after 21 days, whereas S. groenlandicus levels after 3 days were twice as high as M. truncata and decreased thereafter to a 21-day value of ~5 ppm. The Bay 10 experiment resulted in initial (6-hr) levels of petroleum in M. truncata and S. groenlandicus of 13 ppm each. Final levels (21 days) in the animals were 7 and 22 ppm, respectively.

TABLE 11 HYDROCARBON CONCENTRATIONS IN TISSUES OF AfYA *TRUNCATA* (MYA), *SERRIPES GROENLANDICUS* (SER), AND *STRONGYLOCENTROTUS DROEBACHIENSIS* (STR)

			µg/g wet weight						
Exposure Conditions[a]	Time	Species	ΣAB	N	ΣC 1N	ΣC2N	ΣC3 N	Total Aromatic	Total Oil
0	6 h	MYA	0,08	0.001	0.001	0.004	0.050	8.9	24
		SER	nd	nd	nd	nd	nd	nd	nd
		STR	nd	nd	nd	nd	0.093	17	44
0.5	6 h	MYA	nd	nd	nd	nd	0.028	5.0	13
		SER	nd	nd	nd	0.011	0.032	5.7	15
		STR	nd	nd	rsd	nd	0.116	21	55
5	6 h	MYA	0.36	0.017	0.120	0.267	0.269	48	130
		SER	0.64	0.024	0.194	0.457	0.455	81	217
		STR	nd	0.005	0.063	0.059	0.137	25	65
50	6 h	MYA	0.04	0.009	0.271	0.701	0.755	135	360
		SER	0.12	0.0 !	0.33	0.91	0.76	136	360
		STR	3.5	0.03	0.34	0.56	0.33	59	160
100	6 h	MYA	nd	nd	0.10	0.61	0.50	89	240
		SER	nd	0.1	0.21	0.60	0.37	66	180
		STR	nd	0.01	0.22	0.12	0.06	11	29
250	6 h	STR	nd	nd	0.03	0.03	0.04	7.7	21
250 (cv,lg)	6 h	MYA	nd	nd	0.23	0.53	0.38	67	1so
		SER	nd	nd	0.26	0.67	0.44	79	210
		STR	nd	nd	0.01	0.09	0.04	7.0	19
250 (cv,sml)	6 h	SER	nd	0.01	0.12	0.22	0.12	22	59
		STR	nd	nd	0.70	0.10	0.08	14	37
500	6 h	:MYA	0.05	0.01	0.54	1.9	1.5	270	710
		SER	1.7	0.16	1.2	2.1	1.1	200	540
		5TR	0.5	0.05	0.30	0.20	0.06	11	31
0.5/5/0.2	18 h	MYA	nd	nd	nd	nd	0.03	5.9	16
		SER	nd	nd	nd	0.04	0.27	48	130
		STR	nd	nd	0.04	0.06	0.04	7.9	21
	7 d	MYA	0.01	nd	0.01	0.02	0.10	17	45
		SER	nd	nd	nd	0.01	0.05	8.4	22
		STR	nd	nd	nd	0.18	0.45	81	220
10/1 00/5	18 h	MYA	0.03	0.36	0.42	1.6	1.3	240	630
		SER	nd	nd	0.26	1.2	1.1	200	530
		STR	nd	nd	0.11	0.48	0.32	56	1.50
	7 d	MYA							
		SER	nd	nd	nd	0.10	0.31	55	150
		STR	0.08	0.01	0.24	0.88	1.1	190	510
10/100/5 (cv)	18 h	MYA	1.0	0.05	0.58	1.7	1.1	200	540
		SER	0.39	0.05	0.05	0.18	0.20	37	99
		STR	2.7	0.12	0.58	0.52	0.13	23	61
	7 d	MYA	0.07	nd	0.32	0.3 1	0.72	130	340
		SER	nd	0.10	0.20	0.10	0.36	65	I 70
		STR	nd	nd	0.08	0.48	0.80	140	3s0
10/100/5	18 h	MYA	2.7	0.11	0.73	2.0	1.2	210	570
		SER	0.03	0.01	0.43	1.9	1.8	320	860
		STR	2.2	1.0	0.50	0.56	0.24	43	120
	7 d	MYA	ND	ND	ND	0.01	0.10	18	47
		SER	ND	ND	ND	0.01	0.07	12	33
		STR	0.80	0.08	0.15	0.59	0.74	130	350

a In order: Exposure concentration (ppm); where indicated, cv specifies tanks were covered during exposure; animal size, large or small where indicated, otherwise medium size range.

nd = not detectable

Levels In S. droebachiensis and compositional uptake patterns were considerably different than those for the filter-feeders in both the Bay 9 and Bay 10 experiments. S. droebachiensis uptake in the Bay 9 experiment did not occur to any significant extent until 36 hours when a level of 3 ppm was observed. Uptake increased to 16 ppm at 3 days and decreased thereafter (9 ppm at 7 days; ~ 2 ppm at 21 days). The Bay 10 experiment resulted in no significant oil uptake by S. droebachiensis, although GC traces did reveal the presence of small amounts of the water-soluble petroleum aromatics.

The chronic exposure (BII) experiments (Table 12) showed suite convincingly that the gut of both M. truncata S. groenlandicus contained most of the petroleum acquired and retained by these animals. In the 50 ppb exposures, the gutted M. truncata and S. groenlandicus animals contained detectable, but small, (~2 ppm) quantities of oil. However, concentrations in the gut analyzed separately were 3-6 ppm in M. truncata and were much higher, 8-60 ppm, in S. groenlandicus. At the 500 ppb exposure level, concentrations in M. truncata (gutted) ranged from 5-7 ppm during the experiment, while levels in the gut were 14-50 ppm. The gutted S. groenlandicus contained 1-20 ppm, while their gut contained much higher levels, 55-400 ppm.

The urchins exposed at 50 ppb and 500 ppb (611 set) showed little oil uptake and their tests contained no detectable oil. GC compositional details are presented in Table 14.

In addition to the gross compositional data on the f_1 (saturates) and f_2 (aromatics) distributions, four parameters based on GC data on the saturate fraction are presented as well (Table 13). These are 5HN, Alk/ISO, Phytane/n.-c_{18} and Pristane/Phytane ratios.

Two sediment samples were also analysed as part of this program. A full GC analysis was performed. Low levels of oil (0.8 ppm) were found in the sediment sample collected from one of the experimental tanks 8 days after the beginning of the experiment, while no oil was found in the control sediment (<.05 ppm). Tissue hydrocarbons were assessed in exposed and control S. groenlandicus (Table 12) and tissues of animals exposed to contaminated sediment contained 7 to 12 ng/g.

The control animals (S. groenlandicus, M. truncata and S. droebachiensis) from Milne Inlet were anal vzed by UV/F. GC. and GC/MS. No petroleum was detected in any of these an reals by UV/F (detection limit 0.05 ppm). GC traces revealed only biogenic hydrocarbon components. GC/MS analyses for the presence of individual aromatics revealed only trace levels 1-3 mg/lg of phenanthrene and methyl phenanthrene in the hree species.

TABLE 12 TOTAL PETROLEUM BY UV/F and GC (ug/g WET WEIGHT)

series	Species	concentration	Time of Sampling: UV 6 Hrs	GC 18 Hrs	UV 36 Hrs	UV 3 Days	GC 7 Days	UV 10 Days	Uv 14 Days	GC 21 Days
Control A	Mya			3.0			3.7			0.62
	Serripes			3.9			3.6			3.6
	Strongylocentrotus			<1			<1			1.0
Bay 9	Mya		65.9	112	48.8	35.8	12.6			10.1
	Serripes		60.3	25.2	115.	89.0	17.5			5.0
	Strongylocentrotus		1.7	<1	3.3	16.0	9.2			2.0
Bay 10	Mya		13.1	30.9	18.8	18.9	7.1			6.6
	Serripes		13.0	50.4	42.4	15.6	21.2			22.2
	Strongylocentrotus		1.4	~1	1.9	1.8	-1			<1
Control B	Mya			<1			<1		1.0	
	Serripes			3.6			2.9		0.46	
	Strongylocentrotus			<1			<1		0.52	<1
BII	Mya	(No Guts) 50 ppb		2.2			<1		2.0	
	Serripes	(No Guts) 50 ppb		2.1			2.0		2.2	
	Mya	(No Guts) 5oo ppb		7.6			4.5		5.2	
	Serripes	(No Guts) 5oo ppb		-1.0			3.0		21.1	
	Mya	(Gut Only) 50 ppb		2.9			5.6		6.4	
	Serripes	(Gut Only) 50 ppb		8.4			57.4		42.2	
	Mya	(Gut Only) 500 ppb		14.0			44.4		49.2	
	Serripes	(Gut only) 500 ppb		55.0			94.4		400.0	
	Mya	50 ppb	2.7			3.4		2.6	-	
	Serripes	50 ppb	2.0			8.1		19.4	-	
	Strongylocentrotus	50 ppb	1.4	<1		1.3	<1	0.76	<1	
	Mya	500 ppb	9.0			12.6		14.4		
	Serripes	500 ppb	10.3			19.8		41.7		
	Strongylocentrotus	500 ppb	2.2	<1		1.1	(1	20.8	2.0	
	Strongylocentrotus (Test)	50 ppb		<1			<1		<1	
	Strongylocentrotus (Test)	500 ppb		<1			<1		<1	
BIV	Serripes (Control A)	30 ppm oil/sed					0.8*			
	Serripes	30 ppm oil/seal					6.7*			
	Serripes	30 ppm oil/sed					12.4*			
	Serripes	30 ppm oil/seal					11.0*			

* 8 Day Samples

GC indicates quantitative analysis performed by GC (See Table 3)

TABLE 14. PETROLEUM HYDROCARBON COMPOSITIONAL PARAMETERS

		Concentration						
nple		f_1 (µg/g Wet)	f_2 (µg/g Wet)	Total f_1 & f_2 (µg/g Wet)	SHWR	ALK/ISO	Pris /Phy	Phy/ n-C_{18}
ntrol A	Mya							
	18 Hr.	1.5	1.5	3.0			60	
	7 Days	3.0	.73	3.7	1.0		14	.62
	Serripes							
	18 Hr.	3.4	0.5	3.9			55	
	7 Days	2.6	1.0	3.6			34	
	Strongylocentrotus							
	18 Hr.	<1	<1	<1			125	
	7 Days	<1	<1	<1			105	
Bay 9	Mya							
	18 Hr.	62.2	50	112	1.7	1.9	1.2	.65
	7 Days	6.4	6.2	12.6	1.1	.40	.27	2.6
	21 Days	3.4	6.7	10.1	1.1		.25	2.7
	W							
		13.1	12.1	25.2	1.1	1.4	1.7	.65
	7 Days	11.5	6.1	17.6	1.1	.13	2.6	7.4
	21 Days	4.1	1.0	5.0	1.1	.10	16.2	1.0
	Strongylocentrotus							
	18 Hr.	1.0	1.0	2.0			61	
	7 Days	1.0	8.2	9.2			113	
	21 Days*	-1.0	20	-3.0			170	1.4
Bay 10	Mya							
	18 Hr.	15.3	15.6	30.9	1.5	1.5	2.0	.69
	7 Days	3.0	4.1	7.1			10.0	3.0
	21 Days	2.8	3.8	6.6	1.6	.21	8.9	2.8
	Serripes							
	18 Hr.	31.4	19.0	50.4	1.8	1.8	1.3	.69
	7 Days	11.7	9.5	21.2	1.0	.25	2.5	10.1
	21 Days	9.8	12.3	22.1	1.2	.14	3.0	8.2
	Strongylocentrotus							
	18 Hr.	<1	<1	-1.0			120	
	7 Days	<1	<1	-1.0			160	
	21 Days	<1	<1	-1.0			85	
ntrol B	Mya							
	18 Hr.	<1	<1	<I				
	7 Days	<1	<1	<1			>200	
	Serripes							
	18 Hr.	<1	<1	<1				
	7 Days	<1	<1	<1				
	Strongylocentrotus							
	18 Hr.	<I	<1	<1				
	7 Days	<1	<1	<1			19	
	Mya (No Guts, 50 ppb)							
	18 Hr.	1.1	1.1	2.2			20.0	.50
	7 Days	<1	<1	<1				
	14 Days	1.0	1.0	2.0				

TABLE 3 (CONTINUED)

Sample		Concentration f_1 (µg/g Wet)	f_2 (µg/g Wet)	Total f_1 & f_2 (µg/g Wet)	SHWR	ALK/ISO	Pris /Phy	Phy/ n-C_{18}
Bll (Cont.)	Serripes (No							
	18 Hr.	1.3	.8	2.1			5.8	.42
	7 Days	1.0	1.0	2.0			3.9	.51
	14 Days	1.0	1.1	2.2			3.3	.77
Bll	Mya (No Guts, 500 ppb)							
	18 Hr.	4.9	2.7	7.6	1.8		5.7	.56
	7 Days	1.3	3.2	4.5	1.2	0.8	3.2	1.3
	14 Days	2.4	2.8	5.2	1.1	1.4	2.8	.65
	Serripes (No Guts, 500 ppb)							
	18 Hr.	<1	<1	1.0				
	7 Days	<1	3.0	3.0				
	14 Days	5.4	15.7	21.1	15	.71	1.3	1.6
	Mya (Gut Only, 50 ppb)							
	18 Hr.	1.0	1.9	2.9				1.6
	7 Days	2.5	3.1	5.6			19.0	2.2
	14 Days	3.0	3.4	6.4			12.0	6.0
	Serripes (Gut Only, 50 ppb)							
	18 Hr.	6.9	1.5	8.4	1.3	1.0	23	1.4
	7 Days	38.4	19.0	57.4	1.2	0.21	3.8	4.4
	14 Days	24.2	18.0	42.0	1.1	0.12	3.4	8.2
	Mya (Gut Only, 500 ppb)							
	18 Hr.	8.5	5.5	14.0	2.0	1.0	18.0	1.0
	7 Days	29.8	14.6	44.4	1.6	.54	5.6	1.3
	14 Days	24.7	24.5	49.2	1.5	.39	3.6	2.2
	Serripes (Gut Only, 5oo ppb)							
	18 Hr.	41.0	14.0	55.0	1.4	.72	3.9	1.1
	7 Days	55.2	39.2	94.4	1.5	.26	2.0	6.6
	14 Days	260.	140.	400.0	1.9	.55	1.1	3.1
	Strongylocentrotus (50 ppb)							
	18 Hr.	<1	<1	<1				
	7 Days	<1	<1	<1				
	14 Days	<1	<1	<1				
	Strongylocentrotus (500 ppb)							
	18 Hr.	<1	<1	<1				
	7 Days	-1.0	-1.0	- 2.0			-	
	14 Days*	-1.0	~1.0	-2.0			57	1:7
	Strongylocentrotus (Test, 50 ppb)							
	18 Hr.	<1	<1	<1				
	7 Days	<1	<1	<1				
	14 Days	<1	<1	<1				
	Strongylocentrotus (Test, 500 ppb)							
	18 Hr.	<1	<1	<1				
	7 Days	<1	<1	<1				
	14 Days	<1	<1	<1				

Discussion

Exposure Concentration:

Throughout this study the choice of exposure concentration was predicated by the desire to encompass water concentrations of dispersed Lago Medio crude as observed in the 1981 field spill. This condition was met by the range of single exposure tests.

A greater similarity with the field scenario was achieved with the three-phase sequential dosing regimes. Similar levels and sequences reported by Green et al (1982) for the 18 h period following the dispersed oil spill of Bay 9 were reproduced by the 10/100/5 ppm concentration series. The 0.5/5.0/0.2 ppm concentration series approximated the 18 hours post-spill levels observed in Bay 10, a site adjacent and down-stream from the dispersed oil spill site. The flushing with clean seawater after 18 hours of sequential exposure in both Bay 9 and Bay 10 simulations matched the trace and undetectable hydrocarbon levels recorded in the field after a similar interval. Repetition of the Bay 9, Bay 10 sequences verified the consistency in behavioral response between years and provided additional support to the 1981 field results. Simulation of prolonged low level release from an leaking tanker or a ruptured pipeline or possibly simulating a dispersed point-source spill in shallow waters was achieved through the long-term 50 ppb and 500 ppb dosing regimes.

Additional information on the observed behavioral differences between the bivalves and particularly on the extent to which accumulated hydrocarbons affect their physiology was obtained from a complimentary study of the filtration, respiration rates and assimilation ratios of the bivalves exposed to chronic and sequential dispersed oil concentration.

A. Behavioral Responses

The behavioral responses shown by the test species exposed to the range of oil concentration described above will be discussed in the context of oil toxicity effects and their significance to the survival potential of the oiled organisms. The relevance of these findings to the iterpretation of the observations and conclusions from the 1981 field oil spills will also be discussed.

The quantification of the behavioral changes in this study emphasized ET 50 values and time to recovery of pre-exposure behaviour. Both 1982 and 1983 ET 50 and recovery values resulted from a best fit curve. Other behavioral changes occurred but either did not affect 50% of the test population or where sporadic in occurrence. These behavioral indices will be described to complement the quantifiable indices tested in Table 2.

Mya truncata continued to be a difficult species for an oil effect assessment. The extreme sensitivity of the test species to external disturbances other than oil and the lack of obvious trend in the behavioral responses to oil was confirmed in 1983. Clear dose responses could not be discerned using the chosen indices. Higher concentrations were more effective in provoking behavioral changes and in delaying recovery times. The tendency of this bivalve to close ostia rapidly at concentrations above 10 ppm may represent an attempt to arrest water flow, filtration and oil input. This would constitute a survival advantage if exposed to oil and seawater. The lack of response to the 50 ppb and 500 ppb chronic exposure may indicate a tolerance level for low hydrocarbon concentrations.

The loss of responsiveness to mechanical stimuli when exposed to higher concentrations may be considered as evidence for narcotization. Other possible physiological explanations for this lack of responsiveness include neural control override or muscle fatigue. M. truncata behaviorally was more resistant to oil than either S. groenlandicus or S. droebachiensis.

Serripes groenlandicus was a more consistent and responsive test organism than M. truncata. The wide range of behavioral responses and dose related responses of many of its indices provided valuable information on the survival potential of this bivalve. Both ostial closure the most sensitive index for this species and the mantle/shell gape characteristic of high concentration exposures were critical in determing the degree of exposure of soft tissues to hydrocarbons. The closure of ostia even at low concentrations of oil resulted in the interruption of filtration and therefore limited the input of oil. This explains the lack of appearance of stress indicators at concentrations lower than 50 ppm. The prolonged exposure to 50 and 500 ppb oil concentrations however seemed to affect the bivalve. Although S. groenlandicus exposed to a chronic 50

ppb oil concentration initially responded in accordance with the above behaviour, ostial functions resumed after 2 days and were later suspended at 11 days. At this time the animal emerged from the sediment in a pronounced narcotized state. It is speculated that the internal tolerance levels of s. groenlandicus was reached after 11 days.

Prolonged chronic exposure to 500 ppb significantly affected the bivalves survival potential. Resumption of filtration and respiration activity after the initial shutdown period followed by the emergence and shell gaping at day 12 of the exposure period resulted in maximum contact of oil in the bivalves soft tissues.

This shell and mantle gaping characteristic of the higher concentration exposures and in the latter phases of the Bay 9 sequential exposure is more likely to result from a direct toxic effect of oil. Hydrocarbon narcosis and muscle fatigue were probably the cause of the total lack of stimulus response at this stage.

The sporadic burst of locomotion recorded at higher concentrations also exposed sensitive tissues to oil in the water. This activity was not manifested during the 500 ppb chronic exposure implying pronounced narcosis of the emerged bivalves.

Exposure of soft tissues also resulted from shell damage. The possibility of increased predation on the bivalve because of its emergence from the sediment, shell gaping, and shell damage has to be considered as a potential oil exposure effect on the benthic population.

The decreased capacity to respond normally to tactile stimuli, as shown during exposure, suggests an early narcotizing effect. The latent recurrence of this negative response is more likely to have been of pathological origin.

Behavioral responses of S. groenlandicus during the 1982 and 1983 sequential dosing demonstrated that although the first low concentration elicited similar responses to corresponding single doses, a second exposure at a higher concentration resulted in a markedly greater response than would have been predicted from a single exposure.

This type of sequential exposure and corresponding response was similar to the situation reported for Bays 9 and 10 following the 1982 field spill at Cape Hatt. Emergence and shell/mantle gaping were outstanding behaviour indices. The simplest explanation for the sensitivity of the species to sequential exposures may be that the animal has only a limited capacity to remain shut down, with shells closed. Once this time is exceeded, somewhat longer than six hours, there is no option but to expose soft tissues to ambient oil.

S. droebachiensis an index species sensitive to oil responded to concentrations of 0.5 ppm and above and displayed a wide range of behavioral indices affected by oil. The sequence, onset and recovery time of these behavioral indices demonstrated a fairly consistent dose response.

Debilitation of tube feet functions occurred initially causing loss of protective covering, of the ability to adhere to the substrate and to migrate. The natural consequences of these losses include increased susceptibility to predation, displacement by currents which may result in physical damage and removal from habitat and food source.

Recovery from this debilitation was latent even at low concentrations. This loss of tube foot function may significantly affect the survival potential of urchins.

The equally early loss of response of tube feet manifested by the curling feet and the loss of ability to respond to tactile stimulation, may result from the narcotizing effect of hydrocarbons in water. Distribution of hydrocarbons by the water vascular system of the urchin would generalize the response. Although interruption of water exchange was not verified, observations of the tube feet changes imply such an interruption was likely. Also, throughout the affected period, the peristomal membrance and the periproct regions displayed a sunken and flaccid appearance suggesting an impaired hydrostatic system.

The spinal response observed may be indicative of a neuromuscular effect at least initially narcotic in nature. Spine drooping and spine rigidity alternated with each other and with a more normal radial but not rigid arrangement. While spine dropping may increase the potential for damage and perhaps predation, spine rigidity may counter that effect. The response of assuming a more uniformly round, almost ball-like appearance with rigid spine may however enhance the potential for physical displacement by currents.

It is interesting to note that this behaviour was characteristic of reproductive adults. The occurrence of shedding and the fully ripe gonads of the 1982 urchins indicate that the urchin population was tested during its spawning cycle.

Recovery of the spine system and of responses to mechanical stimulus occurred sooner than did recovery of tube foot responses. This lends support to the suggestion that two different physiological systems were affected by exposure to oil.

Lethality occurred during the 500 ppm single exposure test and to a limited amount during both 1982 Bay 9 simulation sequential tests. Recovery from the 500 ppm exposure was very limited only two of the original population of 20 survived.

The responses to the 1983 sequential dosing correlated well with divers' descriptions of the state of urchins in Bays 9 and 10 within a few days of the BIOS spill. Loss of adherence, narcotization, free movement in currents and a decrease in the number of urchins in the spill area were all reported for that time period.

However, the absence of spine rigidity, the fewer numbers of animals latently displaying spine droop and the absence of tube foot curling in urchins exposed to the Bay 10 sequential dose in 1983 indicate a high threshold for the presence of oil. Differences in the relative sensitivity of 1982 and 1983 populations and their corresponding behaviour responses may be due to differing degrees of maturity of the test population.

Although spawning of S. droebachiensis in this area is believed to occur in the spring (Snow personal communication) it appears 1982 urchins were sexually mature and active at the time of the dosing. Their greater lipid content and peak activity might serve to explain their greater HC load and their greater sensitivity to oil. Considerable numbers of urchins were seen spawning in May of 1981 but no definitive statement can be made as to the 1983 spawning period.

The lack of behavioral response S. droebachiensis to prolonged exposure to 50 ppb reaffirmed the 1982 conclusion that urchin tolerance levels allows for survival in low hydrocarbon contamination waters. The latent debilitation of spinal and tube foot functions and their much greater recovery time when compared to corresponding single dose responses indicate a lowered survival potential in areas of chronic contamination.

Tests of the effects of Corexit at 50 ppm, the amount used in 500 ppm oil dispersions, showed that all three species were responsive to the compound. The dispersant had a narcotizing effect on all three species, usually shortly after exposure. Recovery from all the noted responses for Corexit was rapid, however, and the overall effect of Corexit was much less than that of oil dispersions.

To summarize the behavioral observations have shown that in these three major arctic benthic species, the direct effects of short term (6 h) exposure to dispersed oil are not likely to be fatal at 250 ppm or less. Behavioral changes may predispose these species, particularly S. droebachiensis to increased predation. Removal from habitat is another indirect effect, potentially most important to urchins.

Although the direct toxicity effects resulting from Bay 9 and Bay 10 exposures are not likely to be fatal to the three test species, S. groenlandicus and S. droebachiensis are likely to be more vulnerable to predation.

Chronic exposure to 50 ppb oil concentration was not deleterious to any of the test species. The potential for survival of S. groenlandicus and S. droebachiensis exposed to 500 ppb over a prolonged period of time maybe reduced and result in the disruption of local populations of both these species.

B. Tissue Hydrocarbons

The tissue hydrocarbon component of this study was little emphasized in 1982 and was limited in terms of both sample size and analytical detail. It was evident, however, that all three species accumulated crude oil hydrocarbons.

The two bivalve species showed maximum levels by 6 h or 18 h, corresponding to the end points of exposure regimes. By 7 d, these levels generally had decreased, even to background concentrations in some instances. In urchins, however, a very different response was noted. Samples of tissues taken at 7 d showed an increased accumulation of hydrocarbons. The source of these is likely to have been from residues accumulated on the external body surface which were then absorbed even during the recovery period. This pattern may serve to explain the latent mortalities in the test populations. Further, an emphasis on volatile hydrocarbons such as the water-soluble alkyl benzenes in the 6 h samples may have been a major factor in the rapid early narcosis which was recorded for urchins.

The pattern of accumulation and release observed in these three species is the same as that observed in the field following the experimental spill (Boehm et al., 1982). The actual levels do not and cannot be expected to correlate since sampling times were not the same. The higher values recorded in this study at 6 h as compared to the first post-spill samples taken in the field may be attributed to differences in sampling times. During the tank study, sampling was carried out immediately after the oil exposure, whereas field study samples were taken 1 to 4 d after exposure.

The results of the metabolic study conducted concurrently (Gilfillan and Vallas 1984) have shown that the different filtration rates observed for M. truncata and S. groenlandicus affect the rate at which hydrocarbons are taken up. However differing filtration rates do not appear to affect the deputation rates observed for these bivalves.

In 1983, levels in S. droebachiensis and compositional uptake patterns were considerably different than those for the filter-feeders in both the Bay 9 and Bay 10 experiments.

The urchins exposed at 50 ppb and 500 ppb showed little oil uptake and their tests contained no detectable oil.

An evaluation of the detailed analysis of tissue hydrocarbons carried out for the 1983 program reveals a differential uptake of hydrocarbons among the species as well as a clear indication of biodegradation.

1. SHWR is the saturated hydrocarbon weathering ratio which decreases from ~2.5 in the unweathered oil to 1.0 in weathered oil as n-alkanes ($n\text{-}C_{10}$ to $n\text{-}C_{17}$) are lost mainly by evaporation.

2. ALK/ISO is the ratio of n-alkanes to isoprenoid branched alkanes in the $n\text{-}C_{13}$ to $n\text{-}C_{18}$ range. As oil is biodegraded, n-alkanes in this range are preferentially degraded relative to the isoprenoids. This parameter varies from ~2.5 in undergraded oil to zero in alkane-degraded oil.

3. Phytane/$n\text{-}C_{18}$ is another biodegradation ratio. At low levels of oil in animals, the high relative amounts of natural pristane one of the isprenoids in the ALK/ISO ratio, and present in zooplankton, which represent part of the animal 's food source, interfere with the interpretation of the ALK/ISO ratio. In this case, phytane/$n\text{-}C_{18}$ represents a "cleaner", more easily interpreted ratio. It ranges from 0.65 in Lago Medio oil and increases as $n\text{-}C_{18}$ is degraded.

4. Pristane/Phytane this ratio gives an indication of the amount of biogenic pristane present relative to the petrogenic phytane. This ratio is~ 0.75 in Lago Medio oil to 7200, where levels of oil are nil and pristane is high.

Using these ratios and the data in Tables 12 and 13 it became apparent that:

a) The Control A animals were generally oil-free, although low levels of petrogenic residues were seen in the M. truncata (7-day) samples. Similar profiles were seen in S. groenlandicus control animals.

b) S. droebachiensis Control animals were oil-free. However, trace quantities of water-soluble napthalenes were occasionally present among the large quantities of biogenic hydrocarbons.

c) GC traces of oil in M. truncata indicate that relatively, unweathered (SHWR=1 .7) under-graded (ALK/ISO=1.9; Phy/C_{18}=.65) oil is found in the tissues at 18 hrs. Subsequently, the petroleum components found at 7 and 21 days were both weathered and degraded. An increase in the ALK/ISO and decrease in the Phy/C_{18} ratios both reflect these changes. The aromatic hydrocarbons initially acquired and retained (18 hrs) are comprised of a large quantity of alkylated benzenes and naphthalenes. Subsequently, the lighter aromatics are depurated, leaving alkylated naphthalene, phenanthrene, and dibenzothonphene components.

d) GC traces of oil in S. groenlandicus parallel the findings for M. truncata, although it appears that the saturated and aromatic components found in the 18-hr samples have been weathered more substantially than the residues found in M. truncata.

e) The saturated hydrocarbon GC profiles of S. droebachiensis indicate that quantities of petroleum hydrocarbons acquired are quite low with Pris/Phy ratios remaining quite high. This indicates a predominance of biogenic material. However, the aromatic GC profiles do reveal the presence of naphthalene compounds, characteristic of a water-soluble fraction (WSF) of petroleum. This explains the near absence of saturated petrogenic alkanes in animals exposed to Bay 9 spill simulations.

f) The same compositional trends were observed for animals exposed to the Bay 10 exposure regime. After 21 days, however, substantial quantities of light aromatics (alkyl benzene and napthalene components) remained in S. groenlandicus tissues. This observation parallels the relative greater overall retention of petroleum by S. groenlandicus at 21 days (22 ppm) compared to animals from Bay 9 and M. truncata from Bay 10. The 21-day Bay 10 S. groenlandicus are "typical" in that saturated petroleum residues are biodegraded (ALK/ISO = .14: Phy/C_{18} = 2.8). Water-soluble aromatics are again found at low levels in the S. droebachiensis samples from 18 hrs, 7 days, and 21 days.

g) Control B M. truncata and S. groenlandicus are free of any detectable petroleum contamination.

h) The M. truncata gutted samples (50 ppb) reveal only trace quantities of petroleum aromatics. The same conclusion holds for S. groenlandicus.

i) The gut of these animals did contain petroleum residues (Table 12). M. truncata contained low levels of a degraded petroleum, while S. groenlandicus contained higher levels after 7 days. The low-level uptake of oil at 18 hrs is characterized by active biodegradation (ALK/ISO = 1.0) which becomes quite marked as more oil is acquired ALK/ISO - 0.21).

j) The gutted M. truncata S. groenlandicus samples contained low to moderate hydrocarbon levels. Most of the acquired oil was located in the gut. Biodegraded hydrocarbons were abudnant in the gut. These experiments confirm the hypothesis proposed by Boehm et al. (1982a) that in vivo biodegradation of oil occurs rapidly within the gut of the Cape Hatt benthic bivalves. This biodegradation may have been mediated by microflora in the gut of the animal as opposed to active metabolism by the animals themselves.

k) The S. droebachiensis samples at 50 ppb and 500 ppb exposures contained low levels of petroleum aromatics at the 500 ppb exposure only.

1) The S. droebachiensis Test samples contained no detectable petroleum by GC.

The difference between 1982 and 1983 tissue samples of S. droebachiensis may again be explained by an absence of a gravid condition in the 1983 samples. Gonadal tissue, which may be expected to maximally absorb hydrocarbons, was undeveloped in urchins of that year. Thus the soft tissue of urchins would have formed a poor reservoir for hydrocarbons, as shown by the low uptake. It may be this low uptake potential which explains the minimal systemic toxicity effects noted for sea urchin behaviour in that year.

Comparison with Field Data

Data from 1981 field experiments (Boehm, 1982b) and from the 1983 tank experiments are compared in Table 14. The field data, originally reported on a dry-weight basis have been converted to wet weight by dividing by 7. No major discrepancies are noted in the M. truncata and S. groenlandicus data, both in the concentrations noted in Table 14 or in comparing compositional data from Boehm et al. (1982b) with that in Table 13. Only the Bay 10 S. droebachiensis data appear not to agree in that no oil is reported for the laboratory animals, while moderate amounts of oil appeared in the field animals.

TABLE 14. COMPARISONS OF 1981 FIELD RESULTS TO 1983 TANK STUDIES

	Body Burdens ppm			
	Bay 9		Bay 10	
Species	**Field**	**Lab**	**Field**	**Lab**
Mya				
18 Flours		112		31
24 Hours	17-31		40-53	
7 Days		13		7
14 Days	16-19		19-22	
21 Days		1-0		7
Serripes				
18 Hours		25		50
24 Hours	26-70		40-47	
7 Days		18		21
14 Days	13-23		20-25	
21 Days		5		22
Strongylocentrot us				
18 Hours		<1		~ 1
24 Hours	7		13	-
7 Days	-	9		~ 1
14 Days	34		16	
21 Days		2		<1

from Boehm et al, 1984

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